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Chairman, Academy Foundation—WILLIAM A. DAILY
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* President and Secretary serve as ex-officio members of the Research Grants Committee.

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DAVID S. WOODRUFF	Lilly Hall of Life Sciences Purdue University West Lafayette, IN 47907
FRANK N. YOUNG, JR.	Department of Zoology Indiana University Bloomington, IN 47401

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HARRY G. DAY	Department of Home Economics
	Indiana University
	Bloomington, IN 47401
HOWARD H. MICHAUD	301 East Stadium Drive
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SPECIAL APPOINTMENTS OF THE YOUTH ACTIVITIES COMMITTEE**Indiana Science Talent Search Committee**

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SPRING MEETING

MINUTES OF THE EXECUTIVE COMMITTEE MEETING

April 28, 1978

The meeting was called to order by President Jerry J. Nisbet at 4:00 p.m. in Brooks Hall in the Mary Gray Bird Sanctuary, Connersville, Indiana, with Janice A. Nisbet serving as Secretary *pro tem*. The minutes of the Executive Committee and of the General Session of the Fall 1977 Meeting were approved.

TREASURER'S REPORT

Treasurer Stanley L. Burden presented a financial report for the period January 1 through April 22, 1978, a summary of which follows:

	Academy Accounts	Administered Accounts	Totals
Balance: January 1, 1978	\$6,082.23	\$11,690.11	\$17,772.34
1978 Income	3,080.87	5,500.00	8,580.87
1978 Expenditures	5,385.54	2,190.05	7,575.59
Balance: April 22, 1978	\$3,777.56	\$15,000.06	\$18,777.62

The Treasurer's report also indicated that there are 566 paid-up members, including 263 senior members, on file. The Treasurer's report was approved.

ELECTED COMMITTEE REPORTS

Academy Foundation Committee. William A. Daily, Chairman, reported that the market value of the J.S. Wright Fund had increased from \$442,243 on September 30, 1977 to \$471,934 on March 31, 1978.

Research Grants Committee. Ralph A. Llewellyn, Chairman, reported that 19 proposals had been received and that five were funded in full, while 13 were partially funded; a total of \$4802 was granted. The committee has requested AAAS funding for secondary school students and will work jointly with the Youth Activities Committee to prepare guidelines and process proposals for AAAS-funded projects.

Director of Public Relations. Walter A. Cory, Director, reported on news releases for the Spring Meeting. A questionnaire to solicit information from the membership is being included in copies of the Newsletter.

The reports of elected committees were approved.

PRESIDENTIAL APPOINTIVE COMMITTEES

Academy Representative to AAAS and AAS. Earl A. Holmes reported that Section X has voted a moratorium on AAAS fellows. He also reported on AAS discussions to raise the assessment for state academies from \$0.05 to \$0.10

per member. The AAAS procedure for dispersal of funds for secondary school students is being revised. Holmes recommended that the Academy consider sending a Junior Academy student to AAAS meetings to present a paper.

Youth Activities Committee. Keith Hunnings, Director of the Junior Academy, reported on proposed project activities. Walter A. Cory, Director of the Indiana Talent Search, reported that 13 winners were selected and honored at an awards banquet at the conclusion of the competition. He also reported that most Science Fairs had been held despite the energy shortage and that 24 delegates would attend the National Science Fair competition in Anaheim, California. The Indiana Science Education Fund, Inc., is providing \$1,000 scholarships for 2-4 Indiana Science Fair award winners; the recipients will be selected at Anaheim.

Library Committee. William A. Daily, reporting for the committee, stated that Volume 86 of *Proceedings* has been mailed. The Volume 87 printing requisition, with 800 hard cover copies, has been processed. The State appropriation for *Proceedings* support was \$8,000 for the year.

Program Committee. Robert H. Cooper, Chairman for the Spring Meeting, reported on arrangements for field trips and other aspects of the program. John D. Goodman, Chairman for the Fall Meeting, distributed materials for section chairmen.

Publications Committee. William R. Eberly, Chairman, distributed guidelines and editorial policies for *Proceedings*, prepared by Editor Benjamin Moulton.

Motion: To continue to have an Index Committee whose purpose is to prepare a 10-year Index on schedule. The Editor is to be chairman of the committee, whose members are to be appointed by the Publications Committee.

Seconded and *carried*.

Suggestions for possible special publications for the Centennial Year include revision of *Indiana Scientists*, a history of the Indiana Academy, and separate volumes in Biological and Geological History similar to the Geological Survey.

Invitations Committee. Jerry J. Nisbet reported that Butler University has extended an invitation to host the Academy for the Centennial Year 1985.

Motion: To accept the Butler University invitation for the 1985 Centennial Year.

Seconded and *carried*.

William R. Eberly reported that preliminary planning was under way for the 1979 meetings at Manchester College.

Motion: To accept the dates of October 18-19 for the 1979 Fall Meeting.
Seconded and *carried*.

Science and Society Committee. John Patton reported that this year's special project has been to underwrite the preparation of a report entitled "Policy Recommendations from the Study: Environmental Policy Decisions, A Case Study of PCB's in Indiana." Holcomb Research Institute has prepared this

report with input from the Science and Society Committee. On January 24, 1978, three representatives of the Academy (Jerry J. Nisbet, Donald E. Henderson, and Charles E. Wier) met with Governor Otis Bowen and his staff to express Academy interest in supporting the establishment of a formal Science Advisory apparatus in Indiana.

The reports of Presidential Appointive Committees were approved.

SPECIAL COMMITTEE REPORTS

Biological Survey Committee. A written report from Theodore J. Crovello, Chairman, was distributed. The committee is developing a computerized literature bank, working with the Department of Natural Resources to create a list of rare plant species of the state, working on an update of Deam's "Trees of Indiana," and developing a people-power register.

Emeritus Membership Selection Committee. Robert H. Cooper, Chairman, reported that the following individuals have requested and are eligible for, Emeritus membership status (initial membership year shown in parentheses):

LOLA M. LEMON (1929)

HAROLD D. WEBB (1930)

ROBERT N. MCCORMICK (1931)

Preservation of Natural Areas Committee. James H. Keith, Chairman, reported that the state nature preserves system now contains 46 areas with a total of 7,037 acres. The most recent addition was made on November 16, 1978, with the dedication of the 304-acre Hoosier Prairie Nature Preserve.

Speaker-of-the-Year Committee. John B. Patton, Speaker of the Year, reported that he presented lectures at Indiana State University at Evansville, St. Joseph College, Indiana Geological Survey at Bloomington, Indiana University at Kokomo, Huntington College, Purdue University at Westville, and Indiana University at Bloomington.

Reports of special committees were approved.

The meeting was adjourned at 6:00 p.m.

Respectfully submitted,
ROBERT E. VAN ATTA, Secretary

SPRING MEETING

MINUTES OF THE GENERAL SESSION

April 28, 1978

The meeting was called to order by President Jerry J. Nisbet at 7:30 p.m. in Brooks Hall in the Mary Gray Bird Sanctuary, Connersville, Indiana, with Janice A. Nisbet serving as Secretary *pro tem*.

Erich Klinghammer, Purdue University, presented a fascinating illustrated lecture on "Wolves in Captivity and the Wild."

Stanley L. Burden presented a brief summary of committee reports received by the Executive Committee on April 28, 1979.

Howard R. Youse, Chairman of the Resolutions Committee, presented the following:

RESOLUTION:

WHEREAS: The Indiana Academy of Science is deeply grateful to the Indiana Audubon Society for the use of the Mary Gray Bird Sanctuary and its facilities during Spring Meeting; and

WHEREAS: Mr. and Mrs. Denzel Barricklow, managers of the facilities, have been most helpful in arranging this meeting,

RESOLVED: That the Academy members and guests here assembled express their sincere appreciation to the Indiana Audubon Society for the use of these facilities during this meeting.

We express our thanks to Dr. Goodman and Dr. Cooper, co-chairmen of the Program Committee, who worked with Mr. and Mrs. Barricklow in arranging this meeting.

We wish to thank Professor Erich Klinghammer for his very interesting address on wolves.

We also wish to extend our appreciation to all the leaders of the field trips.

Motion: That the resolution presented by the Resolutions Committee be approved.

Seconded and *carried*.

The meeting was adjourned at 9:30 p.m.

A meeting for Section Chairmen and Chairmen-Elect was held immediately following the General Session. Handouts pertaining to aspects of the 1978 Fall Meeting were distributed from the Secretary, Treasurer, Editor, Director of Public Relations, and the Program Committee.

Respectfully submitted,

ROBERT E. VAN ATTA, Secretary

94th ANNUAL FALL MEETING

MINUTES OF THE EXECUTIVE COMMITTEE MEETING

November 2, 1978

The meeting was called to order by President Jerry J. Nisbet at 7:05 p.m. in Room 101, Hartung Hall at Anderson College, Anderson, Indiana. The minutes of the Executive Committee and of the General Session of the Spring 1978 meeting were approved.

TREASURER'S REPORT

Treasurer Stanley L. Burden presented a financial report for the period January 1, 1978 through October 18, 1978, summarized as follows:

	Academy Accounts	Administered Accounts	Totals
Balance: January 1, 1978	\$6,082.23	\$11,690.11	\$17,772.34
1978 Income	9,382.05	11,592.50	20,954.55
1978 Expenditures	5,629.28	7,799.55	13,428.83
Balance: October 18, 1978	\$9,835.00	\$15,463.06	\$25,298.06

The Treasurer's report listed 769 currently paid-up members, including 416 senior members, with 74 new member applications; 122 members and clubs were dropped for non-payment of dues.

STANDING COMMITTEE REPORTS

Academy Representative to AAAS and AAS. No report.

Auditing Committee. No report.

Youth Activities Committee. Walter A. Cory, reporting for Chairman Donald R. Winslow, stated that the Indiana Science Talent Search and 12 Regional Science Fairs were completed in spite of severe weather problems. The committee recommended that the President of the Academy designate one member of the Youth Activities Committee as *Assistant Chairperson* and another as *Assistant Coordinator of Science Fairs* to better deal with the complexity of committee activities. The recommendation was noted and called to the attention of President-Elect J. Daniel Webster.

Science Fairs Coordinator Karl L. Kaufman reported that four \$1000 scholarships were awarded from among 17 finalists and that distinctive jackets had been presented to Indiana participants in the International Science Fair in California. He further reported that a \$30,000 grant from Lilly Endowment has been received to sponsor a series of 20 teacher-pupil workshops to be held within the largest school corporations throughout the state.

Junior Academy of Science Director Keith Hunnings reported that 14

papers were presented before 75 members at the Junior Academy meeting at Indianapolis and indicated that the Junior Academy is emphasizing the preparation and presentation of papers resulting from student research.

Library Committee. W. A. Daily, reporting for Chairwoman Lois Burton, stated that Proceedings Volume 86 had been distributed to approximately 500 agencies. Thirty-seven new titles have been received by the Library and 151 volumes from the Library's journals have been bound. Use of the Library's materials has increased greatly, due to on-line access through the OCLS data base.

Nominations Committee. Donald J. Cook, Chairman, presented the committee report and made the following motion:

Motion: That the slate of candidates presented by the Nominations Committee be accepted and presented to the membership at the General Session Meeting.

Seconded and *carried*.

Program Committee. John D. Goodman, Co-chairman, presented an interim financial report for committee activities and made several announcements pertaining to operations during the current meeting.

Publications Committee. William R. Eberly, Chairman, reported that no new monographs are currently under consideration, although it was pointed out that the study, "Endangered and Threatened Vertebrates of Indiana," financed by a 1976 Academy grant, may soon be ready for publication. He reported that two commitments for the "Centennial Series" have been made and that others for this series are being considered. The suggestion was made that the Budget Committee might consider establishing an administered account for financing future publications.

Science and Society Committee. Robert E. Henderson, Chairman, reported on the January meeting with Governor Otis Bowen and the Governor's suggestions in response to the Committee presentation. The committee will continue to support legislative efforts to create a science advisory agency. He also reported the committee report, "PCB's in Indiana: A Study in the Transfer of Chemical Information," was completed in June and that copies are now available.

Membership Committee. J. Daniel Webster, Chairman, recognized the decline in membership during the past year. It was suggested that the new membership brochure to be produced next year may be of assistance.

Fellows Committee. Richard L. Conklin, Chairman, presented the committee report and made the following motion:

Motion: That the following persons, recommended by the Fellows Committee, be elected as Fellows of the Academy.

RALPH J. GREEN, JR.

DARRELL W. NELSON

JON R. HENDRIX

BERNARD S. WOSTMANN

WILTON N. MELHORN

HAROLD L. ZIMMACK

ROBERT W. MEYER

Seconded and *carried*.

Resolutions Committee. No report.

Invitations Committee. The report of the committee, transmitted by telephone, was given by Jerry J. Nisbet for Chairman Philip A. St. John. Manchester College and St. Joseph's College will be the sites of the 1979 and 1980 Fall meetings, respectively. The site of the 1979 Spring meeting, scheduled for April 27, has yet to be selected. Offers to host the 1981 and 1982 Fall meetings have been received; the following motion was presented:

Motion: That invitations to host the Fall meetings indicated be accepted:
1981—Wabash College
1982—University of Notre Dame

Seconded and *carried*.

Necrologist. No report.

Parliamentarian. No report.

The reports of the standing committees were approved.

ELECTED COMMITTEE REPORTS

Academy Foundation Committee. William A. Daily, Chairman, presented a very complete and detailed report, summarized as follows:

I. *Foundation Account*

Balance as of September 22, 1977	\$ 320.36
Income as of September 30, 1978	1,189.88
Disbursements to Academy	300.00
Transferred to Principal (9/22/77)	300.00
Cash Balance as of September 30, 1978	910.24

II. *John S. Wright Fund*

Balance as of September 30, 1977	\$ 20,000.00
Income as of September 30, 1978	22,945.94
Cash balance as of September 30, 1978	6,923.40
Disbursements from Invested Income Account:	
Research Grants	5,000.00
Indiana National Bank Fee	2,250.00
IAS Proceedings, Vol. 86	5,500.00
Cash Balance	1,119.34
Balance in Invested Income Accounts:	\$ 36,000.00

III. *Market Value of all Securities* \$546,586.56

Mr. Daily also reported that the committee expects to provide the Academy Budget Committee with a good estimate of anticipated income for 1979.

Bonding Committee. No report.

Research Grants Committee. R. A. Llewellyn, Chairman, reported that 28 research proposals had been received, of which seven were funded in full and 19 in part. A total of \$8,882 was awarded in grants ranging from \$125 to \$1400. In addition, six Secondary School grants funded by the AAAS were awarded.

A new brochure describing the Academy research grant program, along with new guidelines for proposal preparation, will be sent to all members after

the first of the year. The committee expects to consider larger grant requests more favorably in the future.

Editor's Report. Benjamin Moulton, Editor, reported that publication of Proceedings, Volume 87, will be delayed until after January 1, due to the change in publishers, and that the cost of reprints will be 7-10% higher.

Director of Public Relations. Walter A. Cory, Director, summarized current activities of his office and his continuing efforts to place Academy information in local newspapers. Suggestions were offered for bringing Academy activities to public notice, including use of the American Chemical Society "Men and Molecules" taped series.

The reports of the elected committees were approved.

SPECIAL COMMITTEE REPORTS

Biological Survey Committee. Theodore J. Crovello, Chairman, presented a detailed report summarizing the committee activities for the year. Included were reports on the current status of the Literature Project, the Endangered Species Project, the Flora Indiana Project, the Indiana Vascular Plant Atlas, Indiana Trees, Indiana Environmental Impact Statements, Indiana Vegetation Map Project, and the People Power Project. Mr. Crovello outlined current tasks of the committee and briefly discussed the Indiana Natural Heritage Program. He also asked that all interested persons complete the BSC Activity Form and the 1978 Indiana Biota Literature and Activity Survey form which have been prepared by the committee.

Emeritus Member Selection Committee. Robert H. Cooper, Chairman, presented the following persons for Emeritus Membership (initial membership year shown in parentheses):

HILLIS L. HOWIE (1935)

HOWARD H. MICHAUD (1934)

FRANK J. WELCHER (1934)

Motion: That the persons presented be elected to Emeritus Membership. Seconded and *carried*.

Preservation of Natural Areas Committee. James Keith, Chairman, reported that the State Nature Preserves system now contains 49 areas, totalling 7204 acres. Crooked Lake, Spicer Lake, and Fogwell Forest Natures Preserves were dedicated this year. A survey of the Indiana Coastal Zone (Lake Michigan shore line) has indicated 20 sites suitable for consideration as nature preserves.

Speaker-of-the-Year Committee. John A. Ricketts, Chairman, reported that Speaker-of-the-Year Dr. Samuel N. Postlethwait has completed appearances at Indiana University-Purdue University-Indianapolis and at Manchester College. Other engagements are scheduled at Vincennes University and DePauw University, with one date remaining to be filled.

Academy Representative on Indiana Natural Resources Commission. Damian V. Schmelz presented an informative summary of the function and recent activities of the commission.

The reports of the special committees were approved.

NEW BUSINESS

President Jerry J. Nisbet announced that he had appointed a committee to respond to an invitation from the Indiana Department of Public Instruction for the Academy to participate in support of the "Perspectives" program.

Motion: That the Academy support the "Perspectives" program in ways to be developed by the committee.

Seconded and *carried*.

The Budget Committee meeting was set for Saturday, November 18, 1978 in Indianapolis by President-Elect J. Daniel Webster.

The meeting was adjourned at 10:15 P.M.

Respectfully submitted,

ROBERT E. VAN ATTA, Secretary

94th ANNUAL FALL MEETING

MINUTES OF THE GENERAL SESSION

November 3, 1978

The Business Session of the 94th Annual Meeting of the Academy was called to order by President Jerry J. Nisbet at 10:30 a.m. in Byrum Hall at Anderson College, Anderson Indiana.

Dr. Robert H. Reardon, President of Anderson College, welcomed the Academy on behalf of the college.

President Nisbet introduced the Academy Speaker-of-the-Year, Dr. Samuel N. Postlethwait, Professor of Biology at Purdue University. Dr. Postlethwait presented an interesting and inspirational slide-illustrated lecture entitled "Investigating Teaching Alternatives."

The Business Meeting was recessed at 12:00 noon and reconvened at 1:30 p.m. in Byrum Hall.

The Secretary of the Academy presented a summary of committee reports and informed the membership of official actions taken by the Executive Committee on November 2, 1978.

The names of individuals who were elected 1979 Divisional Chairmen and Chairmen-Elect include:

ANTHROPOLOGY

Chairman:	CHARLES P. WARREN
Chairman-Elect:	ERNST VON RAHL

BOTANY

Chairman:	ANNE SUSALLA
Chairman-Elect:	GARY DOLPH

CELL BIOLOGY

Chairman:	MARY F. ASTERITA
Chairman-Elect:	STANLEY N. GROVE

CHEMISTRY

Chairman:	JOHN A. RICKETTS
Chairman-Elect:	EDWARD MILLER

ECOLOGY

Chairman:	H. E. McREYNOLDS
Chairman-Elect:	HERBERT W. SENFT

ENGINEERING

Chairman:	MILTON E. HARR
Chairman-Elect:	RAMACHANDRA A. RAO

(No 1978 election held; 1977-78 officers carry over to 1979.)

ENTOMOLOGY

Chairman: ALAN C. YORK
Chairman-Elect: MICHAEL SINSCO

GEOLOGY & GEOGRAPHY

Chairman: JOHN H. CLEVELAND
Chairman-Elect: KENNETH R. BREHOB

HISTORY OF SCIENCE

Chairman: WILLIAM BLOOM
Chairman-Elect: PATRICK H. STEELE

(No 1978 election held; 1978 officers carry over to 1979.)

MICROBIOLOGY & MOLECULAR BIOLOGY

Chairman: CARL E. WARNES
Chairman-Elect: DONALD A. HENDRICKSON

PHYSICS

Chairman: RALPH A. LLEWELLYN
Chairman-Elect: GERALD P. THOMAS

PLANT TAXONOMY

Chairman: DONALD L. BURTON
Chairman-Elect: JOHN BACONE

SCIENCE EDUCATION

Chairman: H. MARVIN BRATT
Chairman-Elect: WILLIAM G. WERT

SOIL & ATMOSPHERIC SCIENCES

Chairman: GARY C. STEINHARDT
Chairman-Elect: DONALD P. FRANZMEIER

ZOOLOGY

Chairman: RICHARD O. MCCrackEN
Chairman-Elect: ROBERT R. PINGER

The Secretary presented the following motions:

Motion: That the individuals who have applied for membership in the Academy be elected to the types of memberships for which they have applied.

Seconded and *carried*.

Motion: That the individuals recommended by the Emeritus Member Selection Committee and approved by the Executive Committee be elected to Emeritus Membership.

Seconded and *carried*.

Motion: That the individuals recommended by the Fellows Committee and approved by the Executive Committee be elected as Fellows of the Academy.

Seconded and *carried*.

Faye Kenoyer Daily presented the Necrologist's report, which included the names of five members:

GLENN G. BARTLE
LLOYD BEESLEY
W. C. GUNTHER

PAUL E. KLINGE
EDWARD L. HAENISCH

Theodore Crovello presented a brief report on activities of the Biological Survey Committee; he announced that printed copies of the committee's detailed report were available. He also offered the suggestion that a Spring Meeting Symposium might be considered.

Robert Henderson reviewed the activities of the Science and Society Committee and commented on the report, "PCB's in Indiana: A Study in the Transfer of Chemical Information," completed during the past year.

Donald J. Cook, Chairman of the Nominations Committee, placed the following slate in nomination:

President: J. DANIEL WEBSTER, 1979
President-Elect: ROBERT E. HENDERSON, 1979
Treasurer: JOHN A. RICKETTS, 1979-81
Director of Public
Relations: WALTER A. CORY, JR., 1979-80
Academy Foundation: WILLIAM A. DAILY, 1979-80
Bonding Committee: BARRY C. KNISLEY, 1979-80
DONALD R. WINSLOW, 1979-80
Research Grants Committee: ERNEST E. CAMPAIGNE, 1979-83

No nominations were made from the floor.

Motion: That the slate presented by the Nominations Committee and approved by the Executive Committee be declared elected.

Seconded and *carried*.

Richard Conklin, Chairman of the Fellows Committee, presented certificates to those newly-elected Fellows of the Academy who were present.

Howard Youse, Chairman of the Resolutions Committee, presented the following:

RESOLUTION:

WHEREAS: A major goal of the Indiana Natural Heritage Program is to determine what natural biotic communities, species, and other elements of the State's natural heritage are, or may become, endangered and should be preserved; and

WHEREAS: A major means to accomplish this goal is to accumulate information on possible endangered natural communities and species; and

WHEREAS: A major project of the Biological Survey Committee of the Indiana Academy of Science is the development and use of computerized information retrieval systems about the occurrence of all species and other natural elements throughout the State, and of the relevant literature; and

WHEREAS: The amount of work to be done both on an immediate and long-term basis is great, and requires the integrated cooperation of all workers; then

RESOLVED: That the Indiana Academy of Science, in support of the Natural Heritage Problem, endorses continued integrated cooperation between its Biological Survey Committee and the Indiana Natural Heritage Program.

RESOLUTION:

WHEREAS: The Indiana Academy of Science is deeply grateful to Anderson College for their invitation to hold its 94th annual meeting on their campus; and

WHEREAS: Administration, faculty, and students alike have cooperated in providing us their facilities for this meeting; be it

RESOLVED: That the Academy members here assembled express their sincere appreciation to President Dr. Robert H. Reardon for all the courtesies that Anderson College has extended to the Academy during the meeting.

We are especially grateful to Dr. John D. Goodman and his committee for the arrangements of the entire program and the comfort and conveniences provided the membership. We also express our sincere thanks to Dr. Sam N. Postlethwait, Indiana Academy Speaker of the year, for his address, *Investigating Teaching Alternatives*.

Motion: That the resolutions presented by the Resolutions Committee be approved.

Seconded and *carried*.

Steve Justham requested the floor for the purpose of making a presentation. After some discussion, President Nisbet ruled that the proposal must be presented to the Executive Committee prior to General Session action.

The meeting was recessed at 2:15 p.m. and reconvened at 6:00 p.m. with President-Elect J. Daniel Webster presiding at the annual banquet held in Kane Dining Room of the Olt Student Center.

Following introduction of Academy officers and spouses at the speaker's table, Dr. Webster introduced President Jerry J. Nisbet, who presented the annual Presidential Address, entitled "The Brain, the Beauty of Science and Learning."

The meeting was adjourned at 7:25 p.m.

Respectfully submitted,
ROBERT E. VAN ATTA, Secretary

FINANCIAL REPORT

JANUARY 1-DECEMBER 31, 1978

I. ACADEMY ACCOUNTS

	Income	Budgeted	Expenditure	Budgeted
Dues	\$ 7,593.00	\$ 6,640.00		
Reprints: Vol. 86	2,485.96			
87	* 0.00	3,100.00	* 0.00	\$ 3,000.00
Interest	941.58	1,000.00		
Miscellaneous.....	20.00			
Secretary.....			469.24	500.00
Treasurer			320.21	400.00
General Office			169.00	250.00
Travel, AAAS Dues, etc.			194.50	225.00
Membership Committee.....			0.00	200.00
Transfer to Administered Accounts			3,450.00	3,450.00
Junior Academy (\$1,000.00)				
Science and Society (\$500.00)				
Natural Areas (\$0.00)				
Library Binding (\$1,200.00).....				
Proceedings: Publication (\$750.00)....				
Proceedings: Mailing (\$0.00)				
Publications: Clerical				
President's Fund			0.00	100.00
Newsletter			500.00	500.00
Speaker of the Year Honorarium.....			500.00	500.00
Program Committee			***220.30	1,000.00
Publications Editor's Expenses			500.00	500.00
Youth Activities.....			0.00	50.00
Biological Surveys Committee			1,000.00	1,000.00
Representative to AAAS Meeting			300.00	300.00
Public Relations			35.00	100.00
Section Chairman Expenses			0.00	50.00
CPA Fees for Tax Return Preparation ..			0.00	400.00
Lawyer's Fees.....			** 0.00	150.00
Miscellaneous.....			97.45	210.00
	<u>\$11,040.54</u>	<u>\$10,740.00</u>	<u>\$7,755.70</u>	<u>\$12,885.00</u>

* Unexpectedly low because printing of Proceedings Vol. 87 was unusually slow and no billing was received by 12/31/78.

** Unexpectedly low because no billing for tax return preparation was received by 12/31/78.

*** No bills for 1978 Fall Meeting were received by 12/31/78.

II. ADMINISTERED ACCOUNTS

	January 1 Balance	1978 Income	1978 Expenditures	December 31 Balance
Junior Academy	\$ 502.49	\$ 1,000.00	\$ 692.29	\$ 810.20
Science Talent Search.....	272.80	2,050.00	2,157.05	165.75
Science and Society	2,476.29	500.00	244.20	2,732.09
Research	(-3,649.40)	6,057.00	9,468.00	*(-7,060.40)
Natural Areas	0.00	0.00	0.00	0.00
J.S. Wright Library	134.28	0.00	0.00	134.28
Lilly III Library.....	2,632.76	0.00	0.00	2,632.76
Lilly V Library	4,513.20	0.00	0.00	4,513.20
Library Binding	1,196.85	1,200.00	0.00	2,396.85
Science Fairs	0.00	0.00	0.00	0.00
Publications:				
Proceedings	800.89	788.00	00.00	1,588.89
Mailing of Proceedings	779.43	0.00	72.39	707.04
Monographs	1,530.52	328.50	0.00	1,859.02
Natural Features	0.00	0.00	0.00	0.00
Clerical	500.00	0.00	0.00	500.00
	<u>\$11,690.11</u>	<u>\$11,923.50</u>	<u>\$12,633.93</u>	<u>\$10,979.68</u>

* No draws from trust funds were made to allow greater interest earnings. Payments for research grants were made from Academy operating funds. Transfer can be made later if Academy funds become low.

III. SUMMARY

	Academy Accounts	Administered Accounts	Total
Balance: January 1, 1978	\$ 6,082.23	\$11,690.11	\$17,772.34
1978 Income	11,040.54	11,923.50	22,964.04
1978 Expenditures	7,755.70	12,633.93	20,389.63
Balance: December 31, 1978	9,367.07	10,979.68	20,346.75

IV. BANK BALANCES

Upland United Bank, Upland, Indiana	\$ 3,905.19
Great Western Savings and Loan, Los Angeles, California	6,332.42
First Western Savings and Loan, Las Vegas, Nevada.....	10,109.14
	<u>\$20,346.75</u>

V. SUMMARY OF TRUST FUNDS

A. Foundation Account (00430-00-0)		
Income cash balance (1/1/78)	\$ -39.34	
Total dividends and interest for 1978.....	1,497.36	
Disbursements for 1978		
Research grants	\$ 300.00	
Transfer to principal cash	1,000.00	
	<u>\$ 1,300.00</u>	<u>-1,300.00</u>
Income cash balance (12/31/78)	\$ 158.02	\$ 158.02

Principal cash balance (1/1/78)	53.40	
Total receipts for 1978	1,000.00	
Total disbursements for 1978	-1,000.00	
Principal cash balance (12/31/78)	53.40	\$ 53.40
Market value of investments (12/31/78)		\$24,058.30
Total value of account (12/31/78)		\$24,269.72*
*Carrying value is \$25,389.89		

B. John S. Wright Fund (00430-01-9)

Income cash balance (1/1/78)	\$ 3,961.50	
Total dividends, interest, and other income for 1978	21,606.26	
Disbursements for 1978		
INB fee	\$ 2,250.00	
Transfer to 00430-02-8	21,461.09	
Transfer to Principal Cash	0.00	
	\$23,711.09	-23,711.09
Income cash balance (12/31/78)	1,856.67	\$ 1,856.67
Principal cash balance (1/1/78)	210.33	
Total receipts for 1978	20,000.00	
Total disbursements for 1978	-19,995.95	
Principal cash balance (12/31/78)	\$ 214.38	\$ 214.38
Market value of investments (12/31/78)		\$531,568.40
Total value of account (12/31/78)		533,639.45*
*Carrying value is \$335,314.70		

C. John S. Wright Invested Income Account (00430-02-8)

Income cash balance (1/1/78)	\$ 179.69	
Total interest for 1978	2,250.83	
Disbursements for 1978		
Transfers to principal	\$ 2,000.00	
	\$ 2,000.00	-2,000.00
Income cash balance (12/31/78)	\$ 430.52	\$ 430.52
Principal cash balance (1/1/78)	538.91	
Total receipts for 1978	23,461.09	
Disbursements for 1978		
Purchase of investments	\$19,000.00	
Research grants	0.00	
Proceedings, Vol. 87	5,000.00	
	\$24,000.00	-24,000.00
Principal cash balance (12/31/78)	0.00	\$ 0.00
Market value of investments		\$ 41,000.00
Total value of account		\$ 41,430.52

VI. NOTES

Membership Dues:

According to the Treasurer's records, the current status may be summarized as follows:

902 paid (261 member, 444 senior member, 52 student, 8 family member, 24 senior family

member, 18 sustaining member, 2 senior family sustaining, 4 life, 1 honorary, 75 emeritus, and 13 club members).

182 on file from 1977 but not paid for 1978.

110 new members for 1978 (included in above totals).

8 previous members reinstated during 1978 (included in above totals).

122 members and clubs dropped for nonpayment of 1977 dues.

Dues Structure for 1978:

\$ 2.00 for student memberships

5.00 for memberships and club memberships

10.00 for senior memberships

25.00 for sustaining memberships

2.00 additional for family memberships

300.00 for life memberships

150.00-500.00 corporate memberships

50.00-100.00 institutional memberships

1.00 initiation/reinstatement fee (\$2.00 for family membership)

Savings

The treasurer, from the total assets of both Academy and Administered accounts, has maintained sufficient funds in the checking account to pay current bills throughout the year; the remaining funds have been invested in savings certificates.

Certificates redeemed in 1978

None

Certificates current

- *1. (GWSL) \$5,919.10 invested at 6.75% October 1977; 30 September value \$6,332.42; Maturity at April, 1980.
2. (FWSL) \$3,000.00 invested at 6.75% October 1976; 25 December value \$3,494.84; Maturity at April 1979.
3. (FWSL) \$2,000.00 invested at 6.50% April 1978; 25 December value \$2,204.46; Maturity at April 1979.
4. (FWSL) \$1,000.00 invested at 6.50% June 1978; 25 December value \$1,102.46; Maturity at June 1979.
5. (FWSL) \$1,000.00 invested at 6.50% June 1978; 25 December value \$1,102.46; Maturity at June 1979.
6. (FWSL) \$1,000.00 invested at 6.50% June 1978; 25 December value \$1,102.46; Maturity at June 1979.
7. (FWSL) \$1,000.00 invested at 6.50% June 1978; 25 December value \$1,102.46; Maturity at June 1979.

Total	\$16,441.56
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* Fourth quarter report was not received from GWSL before books for 1978 were closed.

Reprints:

Reprint charges to authors for Vol. 84 are being collected with 1 billing outstanding for a total of \$150.48 (William R. Eberly) and will give a net profit to the Academy (1975, 1976, 1977 and 1978) of \$223.81 in excess of printing costs when all bills have been collected. Reprint charges to authors for Vol. 85 are being collected with 1 billing outstanding for a total of \$149.44 and will give a net profit to the Academy of \$225.93 when all bills have been collected. Reprint charges to authors for Vol. 86 are being collected with 5 billings outstanding for a total of \$362.58 and will give a net profit to the Academy of \$290.62 in excess of printing costs when all bills have been collected. No billings have been made for Vol. 87 because printing has not yet been completed.

Attorney Fees:

Ice, Miller, Donadio & Ryan of Indianapolis have been advising the Academy in matters concerning the reprinting and roles of various publications. The executive committee voted at the Fall Meeting of 1973 to delay the transferring of \$4,226.87 for attorney fees concerning the tax

classification problems to the Academy operating funds from the J.S. Wright fund until these funds are needed for operating expenses.

Research Grants:

Funds totaling \$9,468.00 have been awarded to: Galen Weaver (Goshen), Clarence Dineen (St. Mary's), Peter Bretting (I.U.-Bloomington), James E. Shields (Indianapolis), Mohinder S. Jarial (Ball State), Richard Rowlands (I.U.-Bloomington), Timothy J. Decker (Ball State), Robert A. Hunchberger (Ball State), Robert R. Finger (Ball State), Jeffrey E. Ehrenzeller (Indiana State), Lawrence Mordan (Notre Dame), G. Philip Robertson (I.U.-Bloomington), Vincent A. DiNoto (Indiana State), Douglas Soltis (I.U.-Bloomington), James J. Tobolski (I.U. Purdue-Ft. Wayne), Dixon H. Landers (I.U.-Bloomington), Keven Strunk (I.U.P.U.I.), Paul MacMillan (Hanover), Jennifer Lackman & Nevin Longenecker (John Adams High School), Frank Smietana & Richard Flickinger (Paul Harding High School), Paul Stark & Virginia Rhodes (East Noble High School), Wendy Rhodes & Virginia Rhodes (East Noble High School), Lynda S. Rhodes & Virginia Rhodes (East Noble High School), Allen L. Volina & Milton Sprunger (Twin Lakes High School), Sylvia P. Brehm (Indiana State), Paul D. MacMillan (Hanover), K. B. Kipkowitz & T. W. Cutshall (I.U.P.U.I.), Eric R. Johnson (Ball State), John E. Oliver (Indiana State), Greg Brodie (Purdue), James Sweigard (Goshen), Denni M. Zodda (Notre Dame).

Publications:

Sales to date during 1978 have been \$328.00 for Monographs. The total cost of publishing Vol. 87 of the *Proceedings* is unknown as of December 31, 1978 because of late printing but the State of Indiana is expected to pay \$8,000.

VII. BUDGET FOR 1979

The following budget was approved by the Budget Committee in a meeting on November 18, 1978:

Academy Accounts

Anticipated Income

Dues, Initiation and Reinstatement Fees (55 @ \$2, 400 @ \$5, 500 @ \$10, 100 @ \$0) ...	\$ 7,110.00
Interest on Savings.....	1,000.00
Reprint Charges to Authors.....	3,400.00
	\$11,510.00

Budgeted Expenditures

Secretary	\$ 500.00
Treasurer	400.00
General Office.....	250.00
Officer Travel, AAAS Dues	225.00
Membership Committee	250.00
President's Contingency Fund	150.00
Newsletter	500.00
Speaker of the Year Honorarium	500.00
Program Committee	1,300.00
Publication Editor's Expenses (including \$200 for ½ of 2 yr. index)	700.00
Youth Activities Committee	50.00
Biological Surveys Committee (including Endangered Plant Species and Flora Indiana Project Committees)	2,300.00
Representative to AAAS Meeting	300.00
Reprint Charges to Academy.....	3,000.00
Public Relations (including "Men & Molecules" Program for 1 yr.)	250.00
Section Chairmen Expenses	50.00
CPA Fees for Tax Return Preparation	400.00
Lawyer's Fees	100.00

Miscellaneous (including \$110 for library books) .	100.00	
Transfers to Administered Accounts	3,450.00	
Junior Academy	\$ 1,000.00	
Science and Society Committee	500.00	
Natural Areas Committee	0.00	
Library Binding	1,200.00	
Proceedings: Publication	750.00	
Proceedings: Mailing	0.00	
Proceedings: Clerical	0.00	
Special Publications (Centennial)	0.00	
		\$14,775.00
Endowment Funds		
Anticipated Income		
IAS Foundation	\$ 1,500.00	
J.S. Wright Investment Income	21,900.00	
Invested Income Carry-over	36,000.00	
		\$59,400.00
Budgeted Expenditures		
Bank Fee	\$ 2,700.00	
Research Grants (\$12,000—\$350 AAAS)	11,650.00	
Publications		
Proceedings, Vol. 87 (\$15,000—\$8,000 Ind.—		
\$750 transfer)	6,250.00	
Special Publications (\$5,000 for Cen-		
tennial Publication Acct.)	5,000.00	
		\$25,600.00
Restricted Accounts		
Anticipated Income		
Research Grants Committee (AAAS)	\$ 350.00	(part)
Science Talent Search (Tri Kappa)	2,000.00	
Publications	300.00	
Proceedings	\$ 50.00	
Monographs and Nat. Feat.	250.00	
		\$ 2,650.00
Budgeted Expenditures		
Research Grants Committee	\$ 350.00	(part)
Science Talent Search	2,000.00	
Publications	300.00	
Proceedings	\$ 50.00	(part)
Monographs	250.00	(part)
		\$ 2,650.00

Respectfully submitted,

STANLEY BURDEN, Treasurer

We, the undersigned, have audited the Treasurer's records for the Indiana Academy of Science for the year 1978 and have found them to be accurate and in order.

January 19, 1979

TIMOTHY J. BURKHOLDER
WILSON B. LUTZ

THE INDIANA JUNIOR ACADEMY OF SCIENCE

MINUTES OF THE FORTY-SIXTH ANNUAL MEETING

November 3, 1978

The Forty-Sixth annual meeting of the Indiana Junior Academy of Science was held on Friday, November 3, 1978, on the campus of Anderson College, Anderson, Indiana. President Bryon Stephens, of Highland High School, opened the meeting and introduced the candidates for next year's officers. He also introduced Mark Rollen a representative of Anderson College, who welcomed the academy to the campus.

The minutes of the forty-fifth meeting of the Junior Academy were moved not to be read because of a shortage of time.

After the short business meeting, students in both the junior and senior division presented papers on their research. Students in the senior division were also interviewed for "Outstanding Junior Scientist for the State of Indiana." There was also a paper airplane contest and a glass blowing demonstration, for those not involved in any of the above mentioned activities.

A new innovation, the science club polemic was held. A four member team from each school participated in the polemic, which was a written test of both Knowledge and Logic.

After lunch a business meeting took place where elections were held, a new idea for science fiction papers was brought up and awards were presented.

Mark Lagle of Brown County High School read his science fiction story on the Calaban as an example for next years science fiction category.

Mr. Lee Moss, of Lew Wallace in Gary, presented awards to the following people for their achievements in the paper airplane contest:

JUNIOR DIVISION

Third Place

Brad Wolfe, St. John the Baptist, Ft. Wayne

Second Place

Bill Toler, Brown County Jr. High, Nashville

First Place

Bill Finoglier, St. John the Baptist, Ft. Wayne

SENIOR DIVISION

Third Place

Paul Miller, Lew Wallace, Gary

Second Place

Kevin Aodanas, Marquette, Michigan City

First Place

John Brand, New Haven, New Haven

Mrs. Cheryl Mason, of Highland, then presented awards to the following teams for the club Polemic:

JUNIOR DIVISION

Third Place

Farland Jr. High, Farland

Second Place

Brown County Jr. High, Nashville

First Place

Marquette, Michigan City

SENIOR DIVISION

Third Place

Highland High, Highland

Second Place

Franklin High, Indianapolis

First Place

Marquette High, Michigan City

Mrs. Leota Skirvin of Brown County, presented the awards for the Junior Division Papers to the following people:

Third Place

Jill Walicki, St. John the Baptist, Ft. Wayne

Second Place

Lisa Newlin, Paoli Jr. High, Paoli

First Place

Jody Lynn Sibbitt, Paoli Jr. High, Paoli

Mrs. Jane Tucker, Ft. Wayne, presented awards to the following people for Senior Division papers.

BIOLOGICAL SCIENCES

Third Place

Steve West, Paoli High, Paoli

Second Place

Scott Rathgaber, New Haven High, New Haven

First Place

Lynda Rhodes, East Noble High, Kendallville

PHYSICAL SCIENCES

Third Place

David DiGiola, Marquette High, Michigan City

Second Place

Christopher L. Purcell, Marquette High, Michigan City

First Place

Gregory Rondot, Bishop Leurs High, Ft. Wayne

Mrs. Carrol Ritter, of Paoli, presented the awards to the following people for being named outstanding Junior Scientist for the State of Indiana:

Lynda Rhodes—East Noble High, Kendallville

Cheryl Barbati—Highland High, Highland

The traveling trophy for the top club was presented to the following clubs:

JUNIOR DIVISION

St. John the Baptist, Ft. Wayne

SENIOR DIVISION

Marquette High, Michigan City

Next year's meeting is to be held at Manchester College with the following officers: Cheryl Barbati of Highland High School, President; Scott Rathgaber of New Haven High School, Vice-President; and Jamie Sibbitt of Paoli High School, Secretary.

Bryon Stephens adjourned the forty-sixth annual meeting of the Indiana Junior Academy of Science at 2:00.

Respectfully submitted,
CHERYL BARBATI, Secretary

NEW LITERATURE AND WORKS IN PROGRESS ABOUT THE BIOTA OF INDIANA

1978 ANNUAL REPORT OF THE BIOLOGICAL SURVEY COMMITTEE (BSC) OF THE INDIANA ACADEMY OF SCIENCES

BSC Members for 1978:

THEODORE J. CROVELLO, Chairman, Department of Biology
University of Notre Dame, Notre Dame, Indiana 46556
Phone: 219/283-7496 SUVON: Your access code + 3011

JOHN BACONE	Indiana Department of Natural Resources
WILLIAM CRANKSHAW	Ball State University
JAMES GAMMON	DePauw University
JACK MUNSEE	Indiana State University, Terre Haute
VICTOR RIEMENSCHNEIDER	Indiana University, South Bend
JOHN WHITAKER, JR.	Indiana State University, Terre Haute
DAVID WOODRUFF	Purdue University, Lafayette
FRANK YOUNG, JR.	Indiana University, Bloomington

The goal of the Literature Subcommittee of the Academy's Biological Survey Committee is to accumulate and maintain published and unpublished references on the biota of the State. While in the past the BSC Literature Project has presented its findings in conventional printed form, in addition we now simultaneously add these contributions to the Indiana Academy's computerized literature and works in progress register. It provides updated, integrated bibliographies on particular topics in response to specific requests. Contact Professor Crovello for further details, or to submit literature or project information to the data bank. The data bank contains more information about each reference than is presented in this printed summary. Items omitted include: Higher taxa (common names) to which organisms belong; Classes or orders of organisms; Families; Genera or species; Subjects describing each work; and name, address and telephone number of person to contact for further information on each entry.

The following literature references and works in progress were obtained by circulating requests and questionnaires to chairpersons of 69 life science departments throughout the State, and to participants at the Academy's Fall Meeting. Next year's report also will include the results of a search of the computerized files of Bioabstracts from 1969 to the present. The Committee welcomes suggestions on ways to increase the number of contributions to this annual survey, and to increase awareness of its customized computer search capabilities.

Each literature reference or work in progress may contain the following, separated by semicolons: author(s); date; title; citation; and Indiana counties in which the work was performed. If any of these are absent, the contributor did

not provide it. Counties are abbreviated by using their first five letters. The exception is Saint Joseph, which is abbreviated StJoe. An "ALL" in the county field indicates that the work applies to all 92 Indiana counties. A "MANY" indicates the work applies to many counties. In both cases up to five of the most important counties also may be included.

Each citation begins with a letter which has one of the following meanings:

- P: Formal Publication
- I: In Press in a formal publication
- T: Thesis
- O: Other (semi) published work, e.g., park checklists, maps.
- E: Environmental Impact Statements
- W: Work In Progress
- PIAS: In a citation, PIAS refers to the Proceedings of the Indiana Academy of Science.

ATCHISON, G. J., B. R. MURPHY, W. E. BISHOP, A. W. MCINTOSH, and R. A. MAYES; 1978; Trace metal contamination of bluegill (*Lepomis macrochirus*) from two Indiana lakes; P: Trans. Am. Fish. Soc., 106(6), 637-640.

AUSTAD, S. N.; 1978; Mating strategies and the distribution of male aggression in space and time of the bowl and doily spider; T: Purdue—Dr. P.M. Waser; Tippe.

BACONE, J. A. and T. J. CROVELLO; 1978; Endangered and threatened vascular plant species of Indiana; W: DNR, Division of Nature Preserves; ALL.

BAUSS, S.; 1977; A waterfowl survey along the Ohio River from Madison to Vevay; T: B.A. Thesis—Hanover College—Prof. J.D. Webster; Jeffe, Switz.

CHURCHILL, C. M. and H. TAMAR; 1978; Chemotaxis of *Halteria grandinella*; T: Indiana State University, Dr. Tamar; Vigo, Vande, Warwi, Sulliv.

DENNER, M. W.; 1978; Field survey of chemical components and parasites, algae and trees of Posey and Vanderburg counties; W: Department of Biology, Indiana State University Evansville; Vande, Posey.

DOLPH, G. E. and R. L. KIRKPATRICK; 1979; Leaf size variation in Cook's Woods; I: PIAS; Miami.

DORAN, M. J. and R. R. HLAVEK; 1978; Investigations of the fish populations of the Indianapolis Waterway Canal System; W: Marian College Independent Study; Mario.

ERICKSON, H. T. and M. LITTLE; 1979; Cleistogamous and chasmogamous flower production in *Viola papilionaceae* in response to photoperiod; W: Purdue Prof. Erickson; Tippe.

FAHEY, T.; 1977; The biology of *Coprinus radians ozonium* and *oidium* and their interrelationships; O: Indiana State University J.D. Schoknecht; ALL Vigo.

FEUER, W. A. and G. S. BAKKEN; 1978; Thermal ecology of the communal nests of the eastern tent caterpillar, *Malacosoma americanum*, and the fall webworm, *Hyphantria cunea*; W: Indiana State University—Bakken; Vigo.

FINNI, G., J. D. WEBSTER and J. GAMMON; 1978; Environmental impact statements for additional units on PS7 power plants near Princeton and Cayuga.

FRAZER, D. A., and C. M. KIRKPATRICK; 1978; Parental and brood behaviour of emperor geese in Alaska; P: Wildfowl; Gibson, Vermi.

GAMMON, J. R. and M. D. JOHNSON; 1978; Study of biotic community (benthos and fish) of two Indiana creeks. Under EPA contract; W: EPA Grant; MANY Morga.

GOFF, R. J.; 1973; Intraspecific variation and its taxonomic implications in the parasitic mite, *Androlaelaps fahrenheitsi* (Acarina: Laelapidae); T: Ph.D. in progress, Indiana State University, John O. Whitaker, Jr.; MANY, Vigo, Tippe, Newto, Sulli, Clay, Parke, Posey, Marsh, StJoe (in order of decreasing importance).

GOODMAN, J. D.; 1978; Systematics, ecology and parasitology of trematodes and molluscs; W: Prof. J. Goodman, Anderson College; Madis, Others = Southern Indiana Counties.

HALE, A. M.; 1978; Chestnut blight in Porter County, Indiana; W: Valparaiso University, G. Hicks; Porte.

HUNT, L-M.; 1979; Observations of the habits of *Lygaeus Kalmii angustomarginatus* (Hemiptera: Lygaeidae) in Southern Michigan; I: Great Lakes Entomologist.

JOHNSON, G. D., A. W. MCINTOSH, and G. J. ATCHISON; 1978; The use of periphyton as a monitor of trace metals in two contaminated Indiana lakes; P: Bull. Environ. Contam. Toxicol., 19, 733-740.

KLEMME, R.; 1978; An econometric yield response and forecasting model for corn in Indiana; T: Dept. of Ag. Econ., Marshall Martin; ALL.

KNISLEY, C. B. and W. MOORE; 1977; The Cicindelidae (tiger beetles) of Indiana. Part 1. Collections in 1977 from various natural areas; O: Research Report to Dept. of Natural Resources on Research; MANY Porte, Lapor, Jaspe, Brown.

KREKELER, C. H.; 1978; The biota of the Indiana Dunes National Lakeshore; W: School of Public and Environmental Affairs; IU Northwest, Mark Reshkin; Porte, Lake, LaPor.

LISTER, R. M.; 1978; Application of the enzyme-linked immunosorbent assay to detecting viruses in soybean seed and plants; P: Phytopathology. October 78.; Tippe.

MARTIN, M., B. MCCARL; 1978; The economic impacts of the possible withdrawal of pesticides as it relates to a typical Indiana farm; W: Martin, Purdue; MANY.

MAYES, R. A., A. W. MCINTOSH, and V. C. ANDERSON; 1977; Uptake of cadmium and lead by a rooted aquatic macrophyte (*Elodea canadensis*); P: Ecology 58, 1176-1180.

MCINTOSH, A. W., B. K. SHEPARD, R. A. MAYES, C. J. ATCHISON, D. W. NELSON; 1978; Some aspects of sediment distribution and macrophyte cycling of heavy metals in a contaminated lake; P: J. Environ. Qual., 7(3), 301-305.

MORGAN, D. W.; 1978; A study of the effect of the NipSCO Michigan City Generating Station on salmonid migrations in Trail Creek, Indiana—fourth report and final report; E: Notre Dame's ABL for NIPSCO; LaPor.

MUNSEE, J. R.; 1977; *Simithistruma filitalpa* W. L. Brown, an Indiana dacetine ant (Hymenoptera: Formicidae). P: PIAS 86:253-6; Vermi.

PEYTON, T., A. MCINTOSH, V. ANDERSON and K. YOST; 1977; Aerial input of heavy metals into an aquatic ecosystem; P: Water, Air and Soil Pollution 5(4), 443-451.

PINGER, R. R.; 1978; Vector biology of Culicidae (mosquitoes); Delaw Grant.

RIEMENSCHNEIDER, V.; 1978; Computer data bank for storage of floristic data on Swamp Rose and Spicer Lake Nature Preserves; StJoe.

RIEMENSCHNEIDER, V.; 1978; Flora and plant communities of Spicer Lake Nature Reserve; I: PIAS; StJoe.

ROMANO, R. R., A. W. MCINTOSH, W. V. KESSLER, V. ANDERSON, and J. M. BELL; 1977; Trace metal discharges of the Grand Calumet River; P: J. Great Lakes Res., 3, 144-147.

SCHILLING, E. E., JR.; 1978; A systematic study of the *Solanum nigrum* complex in North America; T: Indiana Univ. Charles Heiser; MANY.

SEVER, D. M.; 1978; Female cloacal anatomy of *Plethodon cinereus* and *P. dorsalis* (Amphibia, Urodela, Plethodontidae); P: J. Herpetology 12:397-406; Parke, Montg.

SEVER, D. M.; 1978; Male cloacal glands of *Plethodon cinereus* and *P. dorsalis* (Amphibia; Plethodontidae); P: Herpetologica 34: 1-20; Montg. Parke.

STORMER, F. A., C. M. KIRKPATRICK, and T. W. HOEKSTRA; 1978; Hunter inflicted wounding of white-tailed deer; I: Wildlife Society Bulletin; Marti.

SULLIVAN, J. F., B. R. MURPHY, G. J. ATCHISON, and A. W. MCINTOSH; 1978; Time dependent cadmium uptake by fathead minnows (*Pimephales promelas*) during field and laboratory exposure; P: Hydrobiologia 57(1), 65-68.

SULLIVAN, J. F., G. J. ATCHISON, D. J. KOLAR, and A. W. MCINTOSH; 1978; Changes in the predator-prey behavior of fathead minnows (*Pimephales promelas*) and largemouth bass (*Micropterus salmoides*) caused by cadmium; P: J. Fish Res. Board Can. 35(4), 446-451.

TAMAR, H.; 1978; The movement groups of saltatory ciliates; P: J. of Protozoology, 25, 23A; ALL.

WEBSTER, J. D.; 1978; Winter Bird-population Study #14—Mixed pine and deciduous forest; P: American Birds, 32(1):27-28; Jeffe.

WENTSEL, R., A. MCINTOSH, and G. ATCHISON; 1977; Sublethal effects of heavy metal contaminated sediment in midge larvae (*Chironomus tentans*); P: Hydrobiologia 56(2), 153-156.

WENTSEL, R., A. MCINTOSH, and V. ANDERSON; 1977; Sediment contamination and benthic macroinvertebrate distribution in a metal-impacted lake; P: Environ. Poll. 14, 187-193.

WENTSEL, R., A. MCINTOSH, and W. P. MCCAFFERTY; 1978; Emergence of the midge (*Lironomus tentans*) when exposed to a heavy metal contaminated sediment; P: Hydrobiologia 57(3), 195-196.

WENTSEL, R., A. MCINTOSH, W. P. MCCAFFERTY, G. ATCHISON, and V. ANDERSON; 1977; Avoidance response of midge larvae (*Chironomus tentans*) to sediments containing heavy metals; P: Hydrobiologia 55(2), 171-175.

WILSON, K. S.; 1978; Orchidaceae of Indiana; W: Purdue—Calumet; ALL.

NECROLOGY

FAY KENOYER DAILY, Butler University

LLOYD BEESLEY

Near Cedar Grove, Indiana
April 9, 1911

Cincinnati, Ohio
April 23, 1977

Mr. Lloyd Beesley was born on a farm a mile from Cedar Grove, Indiana, April 9, 1911. He walked to grade school at Cedar Grove and attended White Water High School in Franklin County, Indiana. He could not afford college during the depression and went directly into farming. He had been interested in natural history and wildflowers from childhood, so after meeting a wildflower photographer about fifty years ago, he took up the hobby, too. His farm near Cedar Grove contained some beautiful woodland where he and his wife spent many hours studying and photographing wildflowers and wildlife.

Mr. Beesley's photography was excellent and his work reached the attention of authors and teachers. He and his wife gave illustrated lectures at Ohio and Indiana public schools, colleges, P.T.A. meetings and clubs. His work was used to illustrate lectures and articles in newspapers, magazines and books. Dr. Rickett's *Wildflowers of the U.S.*, Esther Kellner's *Out of the Woods*, William Thomas' *The Swamp and Rivers of the U.S.* are examples of these works.

Mr. Beesley joined the Indiana Academy of Science in 1965 on a family membership with his wife, Adele. That spring, they gave a beautifully illustrated lecture at the general meeting on *Wildflowers of Indiana*. A note in the program explained that Mr. Beesley took the pictures while Mrs. Beesley did the research and lecturing. Several papers were given jointly by them at fall paper sessions, too. They found over 100 species of wildflowers not previously reported for Franklin County, Indiana. These were color photographed. Over 500 species altogether were found in the county. An article in the *Indianapolis Star Magazine*, May 8, 1966, paid tribute to their accomplishments.

Mr. Beesley died April 23, 1977, of leukemia at Christ Hospital in Cincinnati, Ohio. He had enjoyed a good life, was a beloved husband and was well-liked by friends and neighbors. He lived by the Golden Rule.

PAUL ERNEST KLINGE

Indianapolis, Indiana
September 4, 1918

Bloomington, Indiana
April 25, 1978

Mr. Paul E. Klinge was a science educator born in Indianapolis, Indiana, September 4, 1918, of German ancestry. His education was obtained in Indianapolis where he attended Shortridge High School and received an A.B. degree from Butler University in 1939. He also attended Indiana Central College.

His teaching and professional career began in and around Indianapolis. After a brief stint as Library Assistant at Arsenal Technical High School from 1941 to 1942, he began teaching history at Ben Davis High School from 1942 to 1943. He was a biology teacher at Thomas Carr High School 1943 to 1957. In 1957 Mr. Klinge moved to Bloomington, Indiana, to serve as coordinator for school science at Indiana University, a program designed to help teachers better prepare students for college studies. Besides that work, he held a number of other administrative positions at the university through the years as well as being an assistant professor and later professor of education. He was associate director of science in the Indiana University Aerospace Center from 1963 to 1967; assistant dean and later associate dean for undergraduate development 1966 to 1969; assistant dean of research from 1965 to 1968; associate dean for research and advanced studies from 1969 to 1971; executive associate of the Indiana University Foundation from 1963 to 1972; and associate dean for administration for the Bloomington campus from 1969 to 1972. He was assistant to three presidents of Indiana University: Elvis J. Stahr in 1965, Herman B. Wells while he was interim president in 1968, and John W. Ryan from 1972 until 1978 when Mr. Klinge died.

Mr. Klinge received numerous honors and official positions in various organizations. He was a Ford Foundation Fellow from 1953 to 1954 and conducted a high school study institute at Indiana University in 1956. He served as editor of the *American Biology Teacher* from 1954 to 1969 and was president of the National Association of Biology Teachers in 1959 and was given a rare honor when he was made honorary member. He received the National Science Teachers Association outstanding teacher award in 1952 and was regional director in 1954 and on the board of directors in 1956 to 1958. He was a member of the American Institute of Biological Science Executive Committee from 1962 to 1964 and president of a Phi Kappa Phi local alumni chapter in 1960. He was a member on the executive committee of the Biological Science Curriculum Study from 1958 to 1963; consultant to the U.S. office of Education Conference on Gifted Science Students in 1952. He was also fellow of the American Association for the Advancement of Science. He belonged to several other organizations: Kappa Delta Pi, national honorary society; Indiana Historical Society; National Education Association; Academy of Political and Social Science; Central Association of Science and Mathematics Teachers; and Association for Education of Teachers in Science. His research interests were in anthropology and mycology. He was the author of some educational films in biology and

authored or co-authored several books and numerous articles in biology and education. He was editorial director of Compton's *Dictionary of Natural Science* in 1966; co-editor of *Pollution*, published by the National Association of Biology Teachers in 1973; author of *Biology's Bright Future* in the *American Biology Teacher* (1, 1960); and author of *Newer Resources for Improving Instruction in Biology* (from the Department of Health, Education and Welfare Bul. OE 29039, 1963). Mr. Klinge is listed in *American Men and Women of Science*, *Indiana Lives*, *Leaders in Education* and *Who's Who in American Education*.

Mr. Klinge joined the Indiana Academy of Science in 1938. He presented an interesting paper at the fall meeting of 1951 on the potentialities for using physical anthropology or "somatotyping" in the classification of students according to associated temperament. It was hoped that this would be a way to appraise a child in order to improve his public education. From 1954 to 1972 hardly a year passed without Mr. Klinge's service on one or more of the following committees: Junior Academy, Science Education, Youth Activities, Indiana Science Fairs, Relation of the Academy to State and Science and Society. He was the Director of Public Relations from 1969 to 1972.

Paul Klinge died April 25, 1978, in Bloomington, Indiana. His health had deteriorated after October, 1977, when he suffered a heart attack. A statement by Indiana University President John W. Ryan pointed out that Paul Klinge had devoted a lifetime to the improvement of education and praised him as follows: "Those of us who knew him and who had the privilege of working with him will not forget his example of excellence. But more importantly, he will long be remembered for his steadfast service to his host of friends and to Indiana University."

BLANCHE MCAVOY

Mitchell, Indiana
September 11, 1885

Tulsa, Oklahoma
October 22, 1976

Dr. Blanche McAvoy was a retired ecologist and author at her death October 22, 1976. She was born at Mitchell, Indiana, September 11, 1885. She attended public school in Norwood, Ohio. She received an A.B. degree from the University of Cincinnati in 1909, M.A. from Ohio State University in 1912 and a Ph.D. degree from the University of Chicago in 1930. She spent the summer of 1916 at Columbia University.

Dr. McAvoy's professional service began with teaching assignments in Ohio Public Schools from 1909 to 1911. Then she returned to Ohio Public Schools to teach from 1914 to 1921 after serving as teaching fellow at Ohio State University from 1911 to 1914. Most of the schools in which she taught were in suburban Cincinnati.

Dr. McAvoy came to Indiana to teach at Ball State Teacher's College in 1921 where she taught until 1925. In 1925, she was also a teacher at Western College for Women, now an affiliate of Miami University of Ohio. She then went to Illinois State Normal College in 1926 and taught until retiring in 1954 as Professor Emeritus. A scholarship has been established there in her memory.

Dr. McAvoy joined the Indiana Academy of Science in 1921, the year she moved to Muncie, Indiana, to teach at Ball State Teacher's College. She presented three papers before the Academy meetings in 1923 and 1924. These were on *An Ecological View of Wet Waste Land*; lichens from Delaware County, Indiana; new material on teaching botany. She was also interested in cytology and taxonomy of spermatophytes. Dr. McAvoy was a member of our society for 55 years even though she was out-of-state most of the time (Emeritus Member at death).

Other societies to which Dr. McAvoy belonged were: American Association for the Advancement of Science (fellow), American Botanical Society, Ecological Society of America, Illinois Academy of Science, Sigma Xi, Sigma Delta Epsilon, Gamma Beta Upsilon (National Secretary-Treasurer) and Delta Kappa Gamma (local chapter president 1941 to 1943). She is listed in *American Men of Science*, Visher's *Indiana Scientists* and *Who's Who in the Midwest*.

When Dr. McAvoy retired in 1954, she returned to Cincinnati, but soon moved to a retirement home in Tulsa, Oklahoma. At that time, she presented part of her rose collection to the city rose gardens in Ault Park, Cincinnati, where a sign was erected in appreciation of the gift. She grew 300 different kinds of roses and was particularly fond of the old-fashioned varieties.

Dr. McAvoy was 91 years old when she died at Tulsa, Oklahoma. The success of her years is well recorded above. She was modest, kind and extended a helping hand to all.

DARL F. WOOD

North Manchester, Indiana
November 12, 1892

Mishawaka, Indiana
March 17, 1978

Mr. Darl F. Wood was born in North Manchester, Indiana, November 12, 1892. He was educated in this state and graduated from Chester Township, Wabash County, Indiana, High School in 1910. He received an A.B. degree from Indiana University in 1923 and an M.S. degree from Notre Dame in 1936.

Mr. Wood taught mostly chemistry and physics in high school. His teaching experience began in Kosciusko County, Indiana, from 1911 to 1912; continued in Wabash County, Indiana, 1912 to 1913; Syracuse, Indiana, from 1913 to 1915; Burket, Indiana, from 1915 to 1917; Casey, Illinois, from 1917 to 1919; Jerseyville, Illinois, from 1919 to 1921; Hammond, Indiana, 1921 to 1924; and Mishawaka High School at Mishawaka, Indiana, from 1924 to 1959 (36 years).

At Mishawaka, he became Science Department Chairman. He also organized the Mishawaka High School Marching Band in 1925. He was a member of the Masonic Lodge and Ancient Accepted Scottish Rite, recipient of the Boy Scout Beaver Award, and was a ham radio operator since 1919.

Mr. Wood joined the Indiana Academy of Science in 1934 and was an Emeritus member at death. His interests were in Geology, Physics and Chemistry Sections. He was the only sponsor ever listed in the *Proceedings of the Indiana Academy of Science* for the Mishawaka High School Club, member of the Junior Academy, which dropped its membership at his retirement. He was also a member of the Junior Indiana Academy of Science Council for several years. The Mishawaka High Science Club was active and brought exhibits to the Junior Academy meetings.

Mr. Wood died after a long and useful life of a brief illness in Mishawaka, Indiana, March 17, 1978. A living memorial at the Masonic Home in Franklin, Indiana, will be established in his name.

INDIANA ACADEMY OF SCIENCE NEW MEMBERS—1978

- MR. SCOTT ADAMS, 140 Coddington, West Lafayette, IN 47906
 MR. TOM ALDRED, Box 483, Lapel, IN 46051
 MR. BRIAN D. ANDERSON, Department of Biology, University of Louisville
 MR. LLOYD A. ANDERSON, 307 North Pennsylvania Street, Indianapolis Newspapers, Indianapolis, IN 46206
 MR. CURTIS H. AULT, 611 North Walnut Grove, Indiana Geological Survey, Bloomington, IN 47401
 MR. ROBERT L. BALL, Rt. 14, Box 253, Bedford, IN 47421
 MR. STEPHEN D. BARNARD, 635 Bloor Lane, Zionsville, IN 46077
 MISS SUE BECKER, 424 Raines Street, Plainfield, IN 46168
 DR. WILLIAM BERANEK, JR., Holcomb Research Institute, Butler University, Indianapolis, IN 46208
 MR. JAMES C. BIDDLE, North White High School, Monon, IN 47959
 MISS CHARLENE BLACK, 2001 Roosevelt Street, Gary, IN 46404
 MR. R. MICHAEL BRATTAIN, 3206 Longlois Drive, Lafayette, IN 47904
 DR. KENNETH R. BREHOB, 121 Earth Sciences, Notre Dame, IN 46556
 DR. RANDALL E. BROWN, Department of Biology, Huntington College, Huntington, IN 46750
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PRESIDENTIAL ADDRESS

THE BRAIN, THE BEAUTY OF SCIENCE AND LEARNING

JERRY J. NISBET, Ball State University

The human brain, which weighs about three pounds, is probably the most complex structure in the universe. Your brain contains roughly 10 billion nerve cells connected and interconnected with such complexity as to make the most sophisticated man-made computer look like a child's toy by comparison. A cubic inch of brain tissue may contain 100 million cells with connections to 50 thousand other cells.

The superior part of the brain, known as the cerebral cortex, consists of two hemispheres sometimes referred to as the right and left sides of the brain, or more simply as the right brain and the left brain. The two sides of the brain are joined laterally by a large fiber tract of nerve cells called the corpus callosum. Additional fiber tracts connect each hemisphere to the body. Tracing the fiber tracts that go to the body reveals that the right brain controls the motor and sensory aspects of the left side of the body. The right brain also receives sensory input from the left half of each eye. The left brain controls the motor and sensory aspects of the right side of the body and receives sensory input from the right half of each eye.

If you focus on a point centered in front of your eyes, the visual field to the left of this point is transmitted to your right brain while the visual field to the right of the point is transmitted to your left brain. This phenomenon is explained on the basis of the fifty percent crossover of nerves serving each eye. Damage to the right brain may cause impairment of vision in the left visual field and vice versa.

Through experiments on cats, during the early 1950's Meyers and Sperry found that learning achieved through visual input from one eye to one side of the brain is not transferred to the other side of the brain when the optic chiasm and the corpus callosum are sectioned (10). Sperry (10) reported similar observations on callosum sectioned monkeys where he found that learning with one eye had to be relearned when the animal was exposed to the same learning situation but had to use the other eye.

Sperry (10) further observed that animals in which the corpus callosum is cut behave in essentially the same way as normal animals. The severing of the callosum did not produce mental conflict. When the corpus callosum is cut, each side of the brain functions as a separate complete brain and each is capable of perceiving, learning, and carrying on other mental processes independent of the other side. Apparently at any given instant, one half of the brain gains control and the lower centers respond fully. The next instant, the other half of the brain may assume full control of the lower centers. Each half of the brain is essentially

a whole brain with an entire set of integrating centers, and each half is capable of taking over full control of the body (10).

In 1962 Bogen and Vogel, at the White Memorial Medical Center in Los Angeles, surgically separated the two halves of the brain in a human subject. The experimental surgery was performed in anticipation that severing the corpus callosum would relieve the patient of uncontrollable epilepsy. The surgery was successful in alleviating seizures. Since 1962 a number of patients with severe seizures have had the same operation with similar success in alleviating seizures (6).

Gazzaniga and Sperry have conducted continuing observations on these human patients with severed callosums. The patients provided an opportunity to test for unique functions in the left and right brain (5). By controlling input to one hemisphere at a time they observed specialization of functions in each half of the brain. For example, when patients viewed objects flashed in the left visual field, they could find the object with the left hand, but could not describe the object verbally. Pictures of objects flashed in the right visual field could be described verbally but could not be found with the left hand. Also, when an object was placed in a patient's right hand he could describe the object verbally and in writing, but when the same object was placed in the left hand the person could not give a verbal or written description.

You will recall that visual information seen in the left visual field goes to the right brain while visual information seen in the right visual field goes to the left brain. And, that sensory information from the left hand goes to the right brain while sensory information from the right hand goes to the left brain. Testing of the callosum sectioned patients led Gazzaniga and Sperry (6) to the conclusion that sensory information going to the left brain can be described through speech or writing, whereas information received by or generated by the right brain had to be communicated through non-verbal responses. They further discovered that the right brain was incapable of carrying out even the simplest of mathematical problems. However, the right brain was capable of forming complex ideas and of dealing with abstractions, associations, spatial relationships, and generalizations (6).

The work of Gazzaniga and Sperry has added a significant dimension to our understanding of brain functioning in humans. Their work supports the idea that the left and the right halves of the brain are essentially complete brains with comparable sensory and motor centers. However, they also found that each half of the brain specializes in a separate group of higher order mental activities.

The left brain specializes in linear, sequential thought. It attacks problems logically and analytically. It works like a digital computer and moves step by step through problems to come up with answers based upon reason. The left brain houses the linguistic centers and is the site for processing speech, reading, writing, and arithmetic (9). It is in the left brain that ordinary conscious experience is perceived, and it is in this half of the brain that willed action originates (3).

In contrast, the right cerebral hemisphere is the center for imagination and holistic perception. It treats input intuitively and works like an analog computer

jumping from one idea to another in an effort to synthesize concepts and establish patterns (9). The right side of the brain provides our moments of insight. It is also the right brain which perceives meaning in metaphore, in art and in music. And, it is the center for feelings, appreciations, and visuospatial thought. Unlike the left brain which dominates the verbal activities of our lives, the right brain is essentially non-verbal. It sees pictures and conjures images and is the seat for imagination.

During the first four years of a child's life both sides of the brain appear to be able to process all types of input with equal facility. At about age four, however, something happens which initiates specialization (4). As an individual matures, one side of the brain appears to gain dominance over the other side. We have all encountered people who would be classified as left brain dominant individuals. Such people pride themselves on intellectual prowess or the ability to verbalize about historical, literary and scientific information. We also have encountered people who could be classified as right brain dominant individuals. Such people pride themselves on artistic prowess such as their ability to communicate impressionistic or symbolic messages through music, art and metaphore.

The values which Western civilization imposes on both children and adults are values which are largely attained through left brain activities. Our educational system is dedicated to the development of verbal and mathematical skills (7). We have come to believe that problems in the humanities as well as in the sciences can be solved by logical, and sequential analytical approaches. Our emphasis on left brain thinking has obviously paid big dividends, particularly in the fields of business and technology.

In the sciences, as well as most other disciplines, we recognize the necessity for educating the left brain. However, the mental capacities that we prize the most—that elusive something we call creativity, and that fleeting moment we allude to as insight—these are abilities which require right brain development. The scientist admittedly spends much time in making observations, checking variables, collecting and crunching data, and testing predictions. These logic-oriented left brain operations provide only the background for discovery. The imaginative/intuitive right brain is required to do something worthwhile with the accumulated information (1).

The split-brain theory of the human brain provides us with a very useful model for working with educational problems. If we assume that different higher level, mental activities are housed in each side of the brain, we can use the model to analyse our teaching and hopefully gain insights into how we can provide better instruction to both sides of the brain.

When we examine our teaching in light of the model, we recognize immediately that most of our conscious efforts are directed to the left brain—to the mastery of facts and concepts. We also recognize that our highly polished lectures, computer assisted programs, and reading assignments—our teaching methods—are organized in logical, sequential left brain formats. To evaluate student progress we usually require written playback of input—another left brain activity. We pursue each topic within our courses in great detail then

systematically move to the next topic. If we are to cover the material in a respectable manner very little time can be allowed to ponder the greater meanings, or to consider applications, or to investigate possible associations which may exist among the ideas from different disciplines. The amount of knowledge in science is so vast that we can not take the time to pursue these frills. Or can we? Should we?

Using the model again, let us examine the input we are providing to the other fifty percent of our student's cerebral endowment. Whether we are aware of the fact or not, we communicate with the right side of the brain constantly. We do so by the sincerity in our tone of voice, by facial expressions and other forms of body language, by emphasis on words and ideas, by transmitting the feeling of excitement one derives from discovery, by showing the passion we have for scholarship, by displaying open jubilation for success and compassion for failure, by alluding to the satisfactions of knowing, by dealing with the values and importance of our subject matter, and by bringing alive the fascination and intrigue of science. To me, this right brain input can be synthesized into a simple phrase—"the beauty of science."

The attitude which a person develops toward a field of study probably has a greater impact on long term learning than any other single factor. And, the likelihood that a student will put his left brain knowledge to use is heavily influenced by his right brain concern for the subject matter. When you analyse the traits of good teachers, I think you will agree that they have at least two traits in common. One trait is that they have full knowledge of their disciplines. The other is that they inspire students to learn. They know what to teach, and they also know how to transmit excitement, fascination, concern, and feeling for their subject matter. A good teacher consciously or unconsciously provides quality input to both sides of the brain of his students.

Unfortunately, we sometimes communicate relatively ineffective messages to the right brain of our students. For example, we may inadvertantly give students the impression that the objective for mastery of subject matter is to pass tests. Or, because of the pressure of time we may discourage the exploration of ideas and channel student responses into the simple recall of information. Or, if things have not gone well in a faculty meeting, we may even demean or berate our students. Also, I am sure that all teachers have, at one time or another, cut students short with such statements as, "We do not have time to discuss that issue," or "The reason you need to know this is that it is a prerequisite for the next course." Even teachers suffer the frailties of being human and we can not expect them to be capable of providing positive input to the right brain at all times. A significant thing to remember, however, is that when we thwart or subvert the right brain, about the only type of learning that we can expect is short-term learning.

Teachers have learned how to extend and enhance vocal communication through technology. Books represented the first major technological advance in educational communication. And, of course, in more recent years motion pictures, slide projectors, cassette recorders and computers have greatly expanded the teacher's power to communicate. Regardless of the technological device used, the teacher programs the message which is received by students.

Just as with face to face communication, right brain input as well as left brain input is perceived by students who are the recipients of mediated instruction. And, it is the right brain input that largely determines the success of mediated instruction. We note, for example, that the most successful audiotutorial programs transmit the true enthusiasm of the narrator, and the most successful computer programs incorporate techniques which require the learner to interact with the subject matter rather than to deal passively with left brain storage.

The power of multimedia programs to stimulate right brain functions has been demonstrated by several approaches. Two examples of such research applied to science teaching are illustrated by the investigations of Chapdelaine (2) and Ketcham (8). Chapdelaine used a variation of the Osgood Semantic Differential to measure attitudes about biological concepts before and after students participated in multimedia programs that had been designed to develop positive attitudes toward biological concepts. He found that significant attitude changes can be accomplished in relatively short twenty minute programs (2). Ketcham's approach was to measure galvanic skin response of students as they participated in the same multimedia programs. By this technique she was able to determine particular events and combinations of events which caused emotional arousal (8). Research of this type points to some of the teaching techniques which we can use effectively in communicating with the right brain.

Useful long term learning is dependent upon conceptualization of meaning by the learner. This activity requires processing of information by both sides of the brain. The so called affective components are just as important as the strictly cognitive components. The corpus callosum, which physically connects the two sides of the brain, provides the channels through which integration of higher level mental activity takes place. Transmission of neural activity through the corpus callosum is an extremely important part of mental activity (11). During productive thinking, data stored in linear format in the left brain is sampled for pattern fit by the right brain. The synergy of the two brains acting through the corpus callosum produces conscious mental activity.

We need to see the world as we study its' parts. This is the way the mind is designed to function. As new bits of information are supplied to the left brain, the right brain must be enticed to test the new bits of information within holistic settings. The richness of the data bank in the left brain plus the stage of imaginative/intuitive development in the right brain determines the quality of insightful thinking of an individual. We should remember that the right brain is essentially non-verbal. It deals with imagery, feeling, and generalization. It may see, but its' perceptions must be translated into words by the left brain before they can be communicated. This is a difficult task and requires much effort. How do you describe the beauty of a mountain, the roar of the ocean, the howl of the wolf? How do you explain your appreciation of biology or chemistry or physics? How do you compress the significance of what you know into a few concise statements? Difficult, yes, but these simple examples illustrate our most challenging task as teachers. The task of forcing the left brain and the right brain to work together.

Most truly significant accomplishments are developed in stages over long periods of time. And, most great discoveries are the products of individuals who

make unlikely connections through flashes of insight. I contend that our commitment to values, to the formation of positive attitudes, and to the development of creative thought processes should equal our commitment to teaching the subject matter of our respective disciplines.

At this point I need to reiterate that I have been using the model of the split-brain to direct attention to two very significant and different modes of mental activity. Although substantial evidence exists to support the concept that each of these modes is centered in a different half of the human brain, much additional work remains to be accomplished before we can determine with certainty that these centers of activity are situated in the same location in all people. A model is useful when it helps us think through a problem, and this is the value which I place on the split-brain model. The model enables us to extend the principle of isolated functions within the higher centers of the brain, and at the same time the model provides us with a conceptual basis for understanding how these isolated functions are integrated.

The "Brain" is a fantastic structure. We need to nourish it with the "Beauty of Science"—with the fascination of our disciplines, with feeling, compassion, and dedication, and especially with continuing challenge to fit new information into ever changing patterns of the whole. It is the "Beauty of Science" which provides the catalyst for meaning, for discovery, and for creative thought. These are the truly valued outcomes of "Learning."

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ANTHROPOLOGY

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Abstracts

Summer Archaeological Field Excavations at the Worl and Bersch Sites, Wayne County, Indiana. WILLIAM RESEIGH, KELLY COX and B. K. SWARTZ, JR.——Excavations at the Worl Site, Wy-165 (IAS-BSU), an Archaic occupation, and Earthwork 7, Bertsch Site, Wy-45 (IAS-BSU), a Woodland ceremonial complex, both located near Cambridge City, Wayne County, Indiana were conducted by the 12th Ball State University Archaeological Field School. An assemblage of 40 diagnostic artifacts, including 28 points was recovered within the plow zone at the Worl locality. The commonest point type was McWhinney, though Early Archaic forms were also present. No floor surface was found within Earthwork 7 at Bertsch indicating, despite previous reports, it may be a natural formation.

A Havana Tradition Component at the Cooke Site, Parke County, Indiana. CHARLES M. ANSLINGER and Robert E. PACE, Department of Anthropology, Indiana State University, Terre Haute, Indiana 47809.——Test excavations were conducted at the multi-component Cooke Site (12-P-5) in southwest Parke County during August of 1978. The site is located on a sand ridge near the Wabash River, and is known to have been occupied by Middle and Late Woodland, and Mississippian peoples. A series of 12 five by five foot excavation units were spaced to test for midden and subsurface features on the sides and crest of the ridge. A thin midden and 15 pits of varying sizes were encountered. These produced projectile points, scrapers, lamellar flake blades, bifacial blades, milling stones and other lithic tools, along with 2886 pieces of chert debitage, some heat-cracked stone, 874 potsherds, 3 galena cubes, and a rolled copper bead. One burial disturbed by historic activities was encountered. Carbonized fragments of corn were recovered in water separation of floral/faunal material from 2 pits.

The bulk of sherds, cordmarked with coarse crushed stone and sand temper, have limited diagnostic usefulness. However, 14 decorated sherds are similar to Middle Woodland Havana wares of the Illinois River Valley. Neteler Crescent Stamped and Naples Dentate Stamped are best represented, and these are associated primarily with the Fulton Phase, between B.C. 200 and B.C.-A.D. in the Middle Illinois River Valley. A late woodland component is represented by 2 pits containing carbonized corn kernels. One has produced a C-14 date of A.D. 1170 $\pm$ 105 (UGa-2529) and the other a date of A.D. 1325 $\pm$ 50 (UGa-2528).

Survey of the Big Raccoon Creek Valley, Parke County, Indiana. KRISTINA BUTTER and ROBERT E. PACE, Department of Anthropology, Indiana State

University, Terre Haute, Indiana 47809.——A survey of the lower and central Big Raccoon Creek Valley identified 95 sites and 52 cultural components. Twenty one components were Late Archaic, 10 Middle Archaic, 8 each were Early Archaic and Late Woodland, 4 Middle Woodland and 1 Early Woodland. Early Archaic components were represented by Kirk, Thebes and bifurcated clusters of projectile points, with Kirk being most numerous. Bristol Diagonal Notched and Barbee Corner Notched identified Middle Archaic components. Most Late Archaic points were similar to the Faulkner and Matanzas types. Terminal Archaic Riverton points, common along similar streams to the south, were rare.

Early Woodland and Middle Woodland components, few in numbers, are represented by rounded base Adena points, Snyders, and Lowe Flared Base points. Late Woodland triangular points, but not the pentagonal Albee points, were recovered. Most of the points that were recovered are associated with types concentrated south to the Ohio River and beyond. Tools and debitage are of Attica chert, available in the local region. However, small amounts of chert similar to that generally identified as Harrison County, Harrodsburg, Cataract and Burlington, were recovered.

Magnetic Exploration of Indiana Archaeological Sites. RALPH R. B. VON FRESE, Purdue University, West Lafayette, Indiana 47907.——When the magnetization of artifacts contrasts sufficiently with the magnetization of their soil environment, the magnetic method can provide a rapid overview of their distribution which can help optimize the time consuming and expensive effort of excavation. Frequently magnetic artifacts which are encountered at Indiana archaeological sites include iron objects, baked-clay features (hearths, pottery, roof tiles, bricks, burned daub, etc.), wells, pits (fire, refuse, cache, burials, etc.), and structural trenches (building foundations, canals, ditches, stockade trenches, etc.). Iron objects and baked-clay features normally yield strong and readily detectable anomalies in the near-surface geomagnetic field. Hence, the application of magnetometry is routinely warranted when the location and distribution of these artifacts are of archaeological interest. The magnetic detectability of such soil artifacts as wells, pits and trenches, however, is ultimately a direct function of the iron oxide content of the site's soil environment. Thus, the glaciated terrane of the northern two-thirds of Indiana generally is most suitable to magnetic explorations for soil artifacts, because these soils normally exhibit relatively high iron oxide fractions. Magnetic anomaly data affiliated with artifacts can be processed to yield the lateral and vertical distributions, as well as the magnetic susceptibility, volumetric and mass characteristics of the archaeological features. This information, furthermore, is made available without alteration or destruction of the site. A few examples from a magnetic survey of the Ft. Ouiatenon site (12-T-9) illustrate the potential of the method. In general, its major limitation is the lack of ground truth necessary to unravel the often complex anomaly fields which characterize Indiana archaeological occupations. Thus, the collaboration of the archaeologist is the most essential factor in expanding the further usefulness and scope of the method in applications to Indiana sites.

Archaeology without Excavation. HAROLD L. LINKOUS, Department of Sociology and Anthropology, Indiana University-Purdue University at Fort Wayne, Indiana 46805.——This paper reports on a program of research initiated during the spring of 1978 by the Anthropology Department of Indiana University-Purdue University at Fort Wayne. The research focuses on the archaeological resources in northeastern Indiana, a region about which little is known when compared with the rest of the state. The program is in its preliminary stages. It attempts to record archaeological information in a systematic manner, so that in the future, archaeologists will be able to evaluate the nature of archaeological remains in the area.

Data has been collected in two basic ways. First, surface collections have been made during the physical inspection of sites. These have been segregated and stored according to site, with each site being numbered and located on a topographic map. Secondly, private collections found in the area have been photographed, their places of origin being noted on maps. In addition to these sources of data, historical records and previous archaeological works have been examined.

Excavation is neither necessary or desirable in this initial stage of the archaeological program. Eventually we hope to have accumulated enough information to be of real aid in selecting the most important sites for subsequent archaeological excavation. While evidence produced by this research is certainly less precise than that from excavation, it does show the distribution of different types of sites and their relation to natural surface features.

The Role of the Physical Anthropologist in the Central Identification Laboratory. CHARLES P. WARREN, Department of Anthropology, University of Illinois at Chicago Circle, Chicago, Illinois 60680.——As an example of applied anthropology, this is an account of a twenty-one month period of employment as a physical anthropologist with the U.S. Army Central Identification Laboratory in Sattahip, Thailand, subsequent to the ending of the major recent military activities in Southeast Asia. In the laboratory setting, the physical anthropologist is a member of a team of identification specialists, and the assigned duties are numerous and varied. However, the real duties and responsibilities which are demanded of the anthropologist engaged in the identification of human remains during times of disaster are frequently without precedent in the academic and professional experiences of the anthropologist.

Consanguinity in an Indiana Mennonite and Amish Community.¹ JULANNE MCCARTHY, College of Health Related Professions, Wichita State University, Wichita, Kansas.——Calculation of the inbreeding coefficient according to the Sewell Wright procedure requires that all the founding ancestors of a population are known and the relationships of all descendants are traced exactly. When complete geneologies are not available, consanguinity (inbreeding) can be revealed by the study of isonymy (identical surnames). Crow and Mange have shown how this form of demographic data may be used to estimate the

¹Research sponsored in part by the Indiana Academy of Sciences.

inbreeding coefficient of a population. A study of the history of the Mennonites and Amish of Daviess County, Indiana, show that they are one gene pool. They were grouped together for purposes of this study of consanguinity.

Daviess County marriage records dating back 90 years were analyzed for isonymy. They showed an inbreeding coefficient of .0241 for the Mennonites and Amish. This means the average marriage during this time occurred between first cousins once removed and second cousins. Data derived from published church membership lists showed the Daviess County Amish to have an inbreeding coefficient of .0302. Other researchers have published inbreeding coefficients for the Daviess County Amish ranging from .0625 to .0078¹. The present study and the published data show the Mennonites and Amish in Daviess County are one of the most inbred human populations in the world.

Jerger: An Early Archaic Mortuary Site in Southwestern Indiana

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Introduction

The Jerger Site is located in the White River Valley in Daviess County in southwestern Indiana. It is situated upon a sand ridge overlooking what was probably once a marshy area.

The site was found in a cultivated field by Mike Meade and Rick Oberst, residents of the area, and I was shown the material they had collected from the site. They reported cremated human bone, red ochre, points, bifacial objects, drills, and chert debris scattered over an area of about 2500 square feet. In addition, within the limits of the debris scatter they observed 5 dark areas which appeared to be the features from which the material had originated. Similar surface material indicated that another, seemingly isolated, feature (subsequently designated Feature 1) occurred over 100 feet away.

Fieldwork

When I was taken to the site, some 5 years after it had been found, the surface of an area of about 6000 square feet was littered with a large amount of the kinds of material that had been reported to me. At that time no features were noticeable, but it was obvious that the material had come from burial features disturbed by cultivation. I made a surface collection and subsequently conducted an archaeological excavation which lasted 23 days at the site. The excavation was done with a crew varying from 1 to 7 people in the spring and summer of 1977.

The site was excavated in 5 by 5 foot units, and some 500 square feet of it was investigated. The plowzone was removed, and most of it was screened. The underlying soil was examined for archaeological features. Features were photographed, mapped, and removed. Feature fill was screened, and most of it was saved.

Materials from Disturbed Contexts

The following descriptions of materials recovered from the site surface and the plowzone are based upon preliminary examinations. There is a large quantity of material, and it will require more time to go through it thoroughly.

The cremated bone consists of an abundance of small fragments, some of which have red ochre on them. These fragments include both human and animal bones. Some unburned animal bone is also present. A sample of the burned animal bone was examined by William R. Adams of the Department of Anthropology at Indiana University. Animals identified by him include deer, raccoon, dog or coyote, turtle, and turkey.



FIGURE 1. *Point from the surface of the Jerger Site.*

Almost all of the points (Fig. 1) are heat fractured, and many have been discolored by heat. They have a bifurcated base, many of them have basal grinding, and many have serrated blade edges. There is considerable variation in

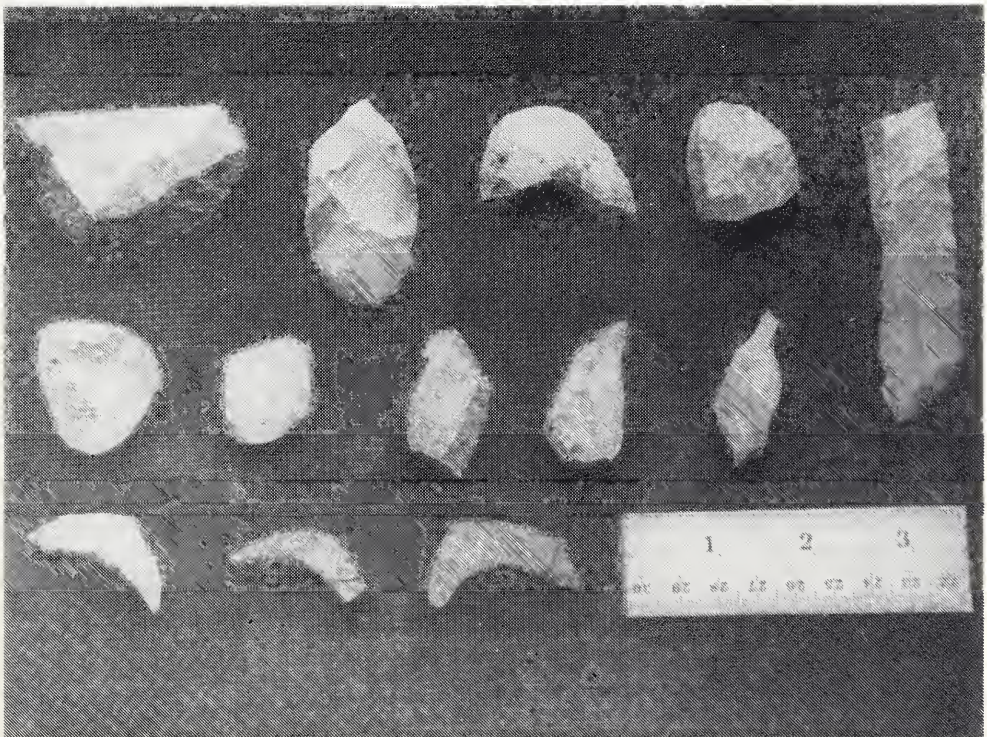


FIGURE 2. *Fragments of bifacial objects from the plowzone of the Jerger Site.*

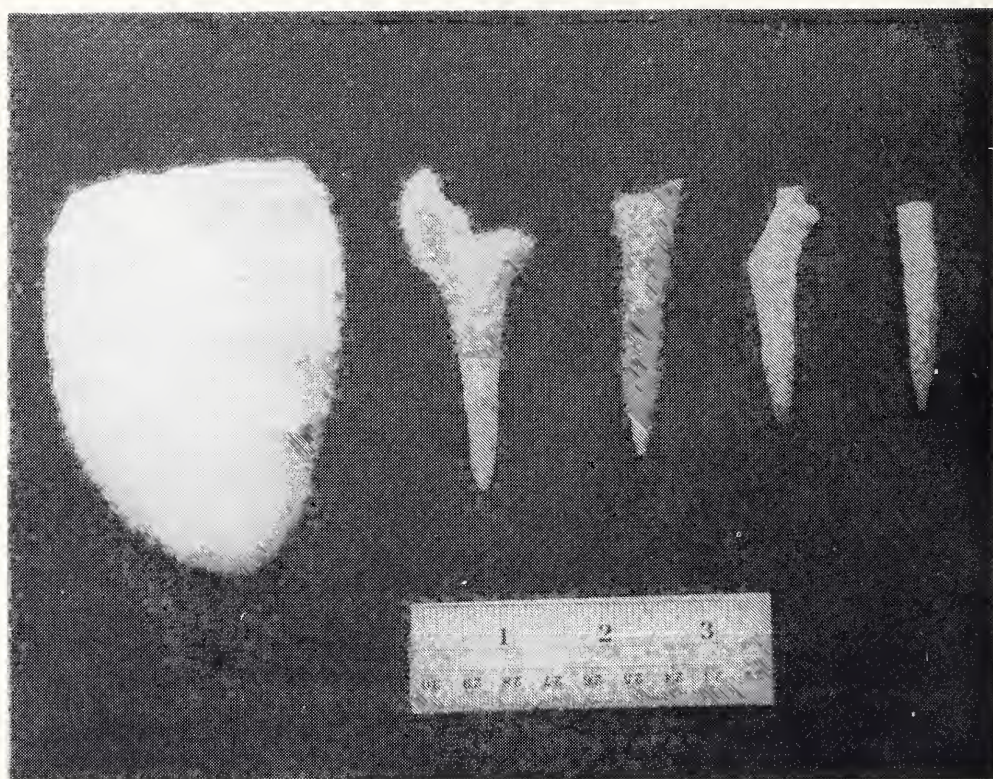


FIGURE 3. *Biface and drills from the area of feature 1 of the Jerger Site.*



FIGURE 4. *Bone artifacts, perforated teeth, and 2 drill fragments from the plowzone of the Jerger Site.*

size. The degree of fragmentation creates measurement difficulties, but to indicate something of the size range there is a variation in length from 38 mm. to well over 65 mm. and a shoulder width range of about 25 to 50 mm.

There are numerous fragments of heat fractured bifacial objects (Fig. 2). They may include parts of preforms, points, and bifaces which are tools in and of themselves. Only 2 complete bifaces have been recovered. One was found in the area of Feature 1 by the discoverers of the site (Fig. 3). It is 102 mm. long, 74 mm. wide, 8 mm. thick, and is well made. It does not appear to have been in a fire. The second was also found on the surface. It is 56 mm. long, 52 mm. wide, and 10 mm. thick. It is a thicker, more roughly made artifact.

A few drill fragments (Figs. 3 and 4) were recovered, and they also exhibit heat damage and alteration. Four (Fig. 3) were found in the area of Feature 1 by the discoverers of the site. Two of them consist of shaft fragments, and 2 are nearly complete. One of the latter has just a small portion of the base intact and has a barbed shoulder. It was at least 60 mm. long. The other has a greatly expanded and rounded base. The shaft is 53 mm. long and 18 mm. at its widest. The base was at least 30 mm. long and at least 31 mm. wide.

Eleven teeth, presumably all animal canines, which have been perforated through the root are present in the collections (Fig. 4). They have been fractured and discolored by heat, and some exhibit polish.

Fourteen fragments constituting the rounded ends of some type of bone artifact have been found (Fig. 4). These fragments are flattened in cross section and are 2 to 4 mm. thick. They vary in width from 6 to 12 mm. and in length from 10 to 20 mm. Their colors (chalky white, gray, and blackish) indicate that they have been exposed to fire. Some similar specimens but broken at both ends were also recovered. These may be shaft fragments of the artifacts with the rounded end.

In addition, a few fragments of other kinds of burned bone artifacts were found. Their functions have not been determined, but some appear to be the remains of awl-like artifacts.

Features

As in the case of the material from disturbed contexts, the discussion of material found in the features is based upon preliminary analyses.

Feature 1 designates a localized and apparently isolated area of surface material. This material is similar to that from the main area of the site, and has evidently been disturbed from an aboriginal feature. We have not excavated in the area of Feature 1.

Three aboriginal features were found during excavation. These are numbered 3, 9, and 13.

Feature 3 was about 6 feet long and 4 feet wide. Its depth from the elevation below plowzone at which it was defined to its bottom was at most about 0.30 foot. Judging from the amount of this feature remaining intact and the quantity of material in the plowzone, Feature 3 has evidently been badly disturbed by cultivation. The fill of the feature was dark and sandy, having a brown to

somewhat of a purplish brown appearance. At the edge of Feature 3, and evidently part of it, was a mottled area about 1 foot in diameter which was stained with red ochre. Hardly any material was found in this area.

Adjacent to the ochre-stained area and to Feature 3 proper was an area about 1.5 feet by 1.0 foot consisting of light tan sand subsoil with some light brown mottling. Hardly any material was found in this area. However, within this area were numerous irregular chunks of cemented light tan sand. These chunks varied in size from pieces about 6 mm. in length to about 35 mm. long. Extending from this area and the ochre-stained area across the central portion of the feature were many such chunks of cemented sand. They were noticeably concentrated near the base of the feature fill and for a depth of about 0.20 foot into the underlying subsoil. Some were also found in the fill of the feature.

The fill of Feature 3 contained material like that from the plowzone. There is cremated bone, a piece of red ochre which may have been shaped, a few point fragments including part of a bifurcated base, some fragments of bifacial objects, 1 of the thin flattened bone shaft fragments mentioned previously, a piece of polished bone, and 5 small shells (Fig. 5).

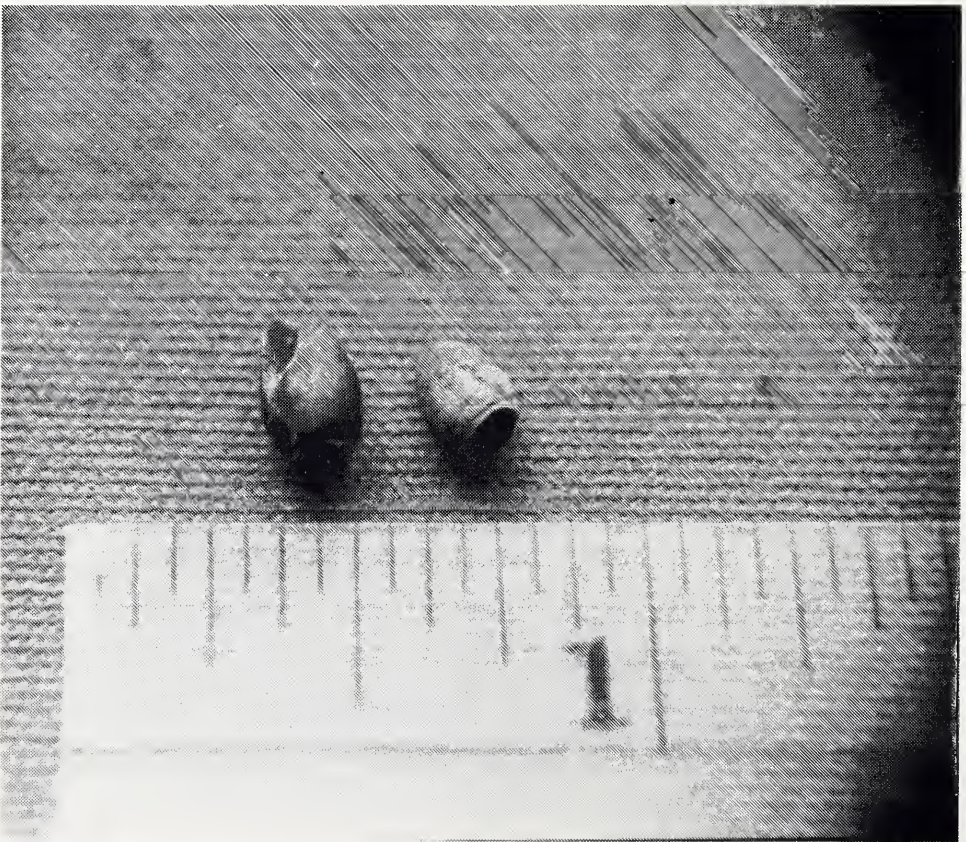


FIGURE 5. *Marine shells from feature 3 at the Jerger Site.*

The shells are black and gray in color indicating their presence in a fire. The largest shell is 8 mm. long and about 4 mm. in greatest diameter. All of them have a broken spire, and they may have been used as beads. These shells were identified by Mr. and Mrs. William B. Adams of Bloomington as marine shells,

possible *Detracia bullaoides*. This is a species found in Florida and the West Indies.

Feature 9 was about 3 feet long and 1.5 feet wide. It extended at most 0.33 foot below the surface at which it was defined. Little remained of this feature as it had been badly disturbed by cultivation. The fill of Feature 9 was sandy and was mottled with browns, the darkest areas having somewhat of a purplish brown appearance. Among the items in Feature 9 were cremated bone and some fragments of points and bifacial objects.

Feature 13 was about 3 feet long and 1.5 feet wide. It extended a maximum of 0.37 foot below the surface at which it was defined. This feature had also been badly disturbed by cultivation. The sandy fill was dark, having somewhat of a purplish brown appearance. The material in Feature 13 included, among other things, cremated bone, some fragments of bifacial objects, and 2 bifurcated base point fragments.

Artifactual Assemblage

The Jerger Site provides evidence for an artifactual assemblage for one of the aboriginal occupations of the White River Valley. It has been known for some time that this occupation existed in the area, being evidenced by the presence of bifurcated base points like those from this site. These points were termed "Category B" and were discussed in a study done several years ago (5). However, until now nothing had been clearly associated with these points in this area.

Based upon my observations and those of the discoverers of the site, little archaeological material occurs at the Jerger Site excepting that originating in the mortuary features. No other kind of aboriginal feature has been found at the site, and it appears that the location was used mainly for funerary purposes. The material from the plowzone is like that found in the features, and material from the two contexts can be associated on the basis of having been burned.

The evidence from this site indicates an artifactual assemblage consisting at a minimum of bifurcated base points, bifacial objects, drills, perforated teeth, bone artifacts with a rounded end, awl-like bone artifacts, marine shells possibly used as beads, and the use of red ochre.

Interpretations

All three of the excavated aboriginal features functioned as graves for the reception of cremations and accompanying items. Since the remains have been in a hot fire and the earth adjacent to the features was not oxidized, cremation evidently occurred elsewhere.

At some point(s) in the mortuary activities red ochre was utilized. It occurs on some of the cremated bone fragments. In addition, a piece of red ochre was recovered from Feature 3, and there was ochre stain in that feature.

The heat-altered points, bifacial objects, drills, perforated teeth, bone artifacts, shells, and animal bone presumably were also burned in the crematory fire(s). Artifacts such as the perforated teeth and the shells may have been worn by the individuals, could have been attached to or been part of an accompanying

object, or have been placed separately. The unworked fragments of animal bone may have been artifactual in concept, e.g. part of a medicine bundle. They may reflect something of the belief system or have been added for some more prosaic reason. It is possible that the treatment of the dead and/or the quantity of and kinds of artifactual accompaniments are indications of status.

Cultural Affiliation and Chronology

Based upon the characteristics of the bifurcated base points recovered, the cultural affiliation of this site is Early Archaic.

The points are similar, at least in concept, to MacCorkle Stemmed, St. Albans Side Notched, and other Early Archaic bifurcated base points from the stratified St. Albans Site in West Virginia (1). Radiocarbon dates place the specimens from St. Albans from about 6000 B.C. to approximately 7000 B.C. Radiocarbon and archaeomagnetic dating indicate similar antiquity for Early Archaic bifurcated base LeCroy and St. Albans Side Notched points at the stratified Rose Island Site in Tennessee (2).

Discussion

The Jerger Site not only furnishes new cultural and paleoecological data regarding a particular Early Archaic population in Indiana, but also provides information which is relevant to this early time period in general.

Knowledge of Indiana's Early Archaic occupations, even artifact assemblages, is quite limited. To my knowledge there is just one other excavated site in Indiana from which there is reasonable indication of an Early Archaic artifactual assemblage. Evidence for this was recovered from and adjacent to a possible living floor buried by alluviation at the Haag Site in Dearborn County in the southeastern part of the State. The Early Archaic assemblage from Haag is thought to include Charleston Corner Notched-like points, bifaces, drills, end scrapers, other unifacial tools, and hammerstones (6).

The Jerger Site provides tangible evidence for the time depth of some kinds of artifacts and cultural practices and furnishes data regarding cultural development in the eastern United States. An important aspect of the Jerger assemblage is that it contributes some information regarding non-lithic artifacts. The presence of marine shells is interesting as an indication of exchange and the use of exotic items as burial accompaniments at this early date.

The Jerger site is one of the few early mortuary sites reported for the eastern United States, and provides increasing indication of the early occurrence of mortuary ceremonialism in the eastern United States. It appears that the Jerger burial features are located in a special cemetery area, and a certain amount of attention, if not special treatment, is indicated by cremation, red ochre, and burned artifacts. The marine shell beads are made from an exotic material and may have special significance. In addition, a sizable quantity of artifactual material accompanied the burials.

At least two other early mortuary sites in the eastern United States which indicate some attention given to the dead are reported in the literature. One is the Renier Site in Wisconsin for which the authors suggest a date of 6500 to 4000

B.C. (3). The Renier burial consisted of cremated bone and fire fractured artifacts including Eden and Scottsbluff points, a side notched point, bifaces, and a scraper. The other site is the Lawrence Site, a multicomponent habitation and burial site in Kentucky (4). Feature 72 contained two individuals accompanied by a Kirk Serrated point, two large points with some Kirk characteristics, a lanceolate point, drills, flake tools, necklaces of dog canines and beaver incisors, several pieces of animal bone, and red ochre. A radiocarbon date of about 5400 B.C was obtained for this feature. The Renier, Lawrence, and Jerger Sites suggest the existence of widespread mortuary ceremonialism at an early date in the eastern United States.

The faunal material recovered from Jerger is important for at least two reasons. It provides data regarding the paleoecology of the area and indicates resource utilization by this Early Archaic population.

In sum, the Jerger Site is significant for a number of reasons, and we plan to continue research and excavation at the site.

Acknowledgements

I wish to express my gratitude to the many people who have assisted me with this project. The site was discovered by Mike Meade and Rick Oberst, and Mr. Meade informed me of the site and took me to it. I am very grateful to both of them for sharing their knowledge of the site with me. Without their aid the excavation would not have been undertaken. I am very much indebted to the landowners, Mr. and Mrs. Gerald Jerger, for permitting excavation and for their many courtesies. Their help is sincerely appreciated. I would also like to thank Craig Kinnaman, Mike Meade, Terry Meade, Rick Oberst, David Sonner, and Garrett Tomak for volunteering their time to help with the excavation. I am especially indebted to Norma Tomak who has helped with all phases of the excavation and analysis. I want to thank Craig Kinnaman for taking photographs of artifacts and of the site. I am grateful to William R. Adams of the Department of Anthropology at Indiana University for examining some of the faunal remains and am grateful to Mr. and Mrs. William B. Adams for examining the shells.

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Abstracts

Anatomy of the Stem of *Arundo donax* L. with Possible Implications for Reed Playability in Woodwind Instruments. MARILYN S. VESELACK and JERRY J. NISBET, Ball State University, Muncie, Indiana 47306.——*Arundo donax* L. has been of significance to the various cultures of the western world because of its role in the development of music. Even today the culms of this plant are still the main source of material used in making reeds for musical instruments.

The goals of our study have been to describe the anatomy of the mature stem *Arundo donax* L., and to investigate possible factors which may be related to the quality of woodwind reeds manufactured from *Arundo* stems. To acquire material for the study, woodwind reeds were obtained that had been used in performance and judged playable by professional musicians. The same musicians were also asked to supply reeds which they judged to be non-playable.

To prepare the material for study, cross sections approximately three millimeters long were sawed from the heel of each woodwind reed. These sawed pieces were then split lengthwise with a knife to make blocks of tissue approximately three millimeters wide. The central blocks of tissue from each reed were softened by chemical treatments and embedded in paraplax for sectioning.

The stem anatomy of *Arundo* is similar to that of corn and other grasses. Under ideal conditions the plant is allowed to grow for 10-20 years prior to harvesting. A prolonged period of natural curing from 1-3 years is recommended prior to using the internodes for the manufacture of woodwind reeds. Apparently much of the *Arundo* crop is currently being harvested after only a few years, and the stems are being kiln dried. Such practices probably have an adverse effect on the quality of woodwind reeds being manufactured today.

Aside from the qualities contributed by fully developed and carefully cured stem material, the proximity of the reed blank to the node, the angle of the blade cut, and the uniformity of distribution of vascular bundles appear to contribute to the playability of woodwind reeds.

The Relationship Between Climate and Growth Habit in the Carolinas. GARY E. DOLPH, Indiana University at Kokomo, Kokomo, Indiana 46901.—— Attempts to relate the leaf form of the total woody flora of the Carolinas with mean annual precipitation and mean annual temperature have not substantiated the predictions of earlier authors. However, not all botanists believe that these

major climatic variables can be related to the leaf form of the total woody flora. Some botanists believe that shrubs are better climatic indicators than trees, whereas others feel that evergreen plants are more sensitive to climate than deciduous. To test these hypotheses, data on the total woody flora of the Carolinas were subdivided in two steps. First, the available data were subdivided into information on trees, shrubs, and vines. Then, each of these categories was subdivided into deciduous and evergreen species. The use of these new data sets did not reveal a better correspondence between leaf form and climate in the Carolinas. Although broad regional correlations do exist, more field work will have to be carried out before the nature of the correlation at the local level can be substantiated.

Notes on *Dryophyllum tennesseensis* Berry (Fagaceae) from the Middle Eocene Claiborne Formation of Tennessee and Kentucky. JAY H. JONES* and DAVID L. DILCHER, Indiana University, Bloomington, Indiana 47401._____The species *Dryophyllum tennesseensis* was established by E. W. Berry in 1916 for a specific leaf type common to the Clairborne Formation of southeast North America. This species is part of a group of leaf types assigned to the genus *Dryophyllum* Debey. Early workers postulated that members of this extinct genus were ancestral to modern genera of the Fagaceae. Ball later concluded that this genus represented a dead end line of evolution which was parallel to modern genera of this family. As part of a study aimed at providing a better understanding of the nature and true position of this problematic genus we have re-examined the species *D. tennesseensis*. This involved detailed leaf architectural and cuticular analyses of many fossil specimens as well as leaves from over 175 extant species of Fagaceae and other families containing similar leaf types. The results of this investigation indicate that the cuticular and leaf architectural characteristics of *D. tennesseensis* are consistent with those commonly observed in the Fagaceae. However, when intrageneric variations are considered this leaf type can not be excluded from the modern genera *Quercus* and *Castanea* of this family. Therefore, based on the analysis thus far completed, we suggest that this leaf type can be confidently assigned to the Fagaceae. However, the question of whether *Dryophyllum* is ancestral to or evolved parallel with modern genera of the Fagaceae can not be determined on the basis of leaf characteristics alone.

Angiosperm Reproductive Organs from Middle Cretaceous Sediments. CAROLYN B. DIEFENBACH and DAVID L. DILCHER, Department of Biology, Indiana University, Bloomington, Indiana 47401, and HOWARD C. REYNOLDS, Fort Hays State University, Hays, Kansas 67601._____Two inflorescence types from the Middle Cretaceous Janssen Clay Member of the Dakota Formation have been examined and found to have a similar morphology. Although the types are from different localities (Russell and Barton Counties, Kansas), both are composed of numerous apetalous florets arranged helically on an elongate or globose axis. Each sessile floret consists of a circlet of four laminar stamens and each individual stamen contains four pollen sacs. While most of these inflorescences appear to be unisexual, other structures, as yet poorly understood, are present on some specimens. These structures may be pistillate and are presently under investigation. *Retimonocolpites* type pollen has been isolated from the florets of well preserved specimens from the Russell County

locality. This pollen is monosulcate with a reticulate sexine that tends to separate easily from the smooth nexine. The pollen grains are found most often as dyads, but single grains and occasional catasulcate tetrads have also been observed. Transmission electron microscope studies of the pollen show the presence of a nonlaminated endexine which may be indicative of angiosperm pollen. The distinctive cuticle of these inflorescences is comparable to cuticle of compound leaves which are also common at both of the localities mentioned above.

Detection of Soil Moisture Variations from Vegetative Soil Cover of Multi-Temporal Landsat Data. STEVAN J. KRISTOF, Laboratory for Applications of Remote Sensing, Purdue University, West Lafayette, Indiana 47907._____A study was conducted to determine the feasibility of using machine-aided processing of Landsat data to observe soil moisture conditions via soil vegetative cover. Multispectral Landsat data were collected over Vigo County, Indiana in 1973 on January 16, May 4, June 9 and September 7. A clustering procedure was applied to divide data into 21 groups of sample points of similar spectral characteristics. The statistics processor computed statistics for each of these cluster groups. Those groups then were used in the pattern classifier to classify each of the data points of the entire county into one of 21 spectral classes. The results of classification were compared with ground observations. This study has confirmed that the Landsat data taken at different times of the year are influenced by the sun elevation. The radiations are smaller in the Spring than in the Summer. Relative values representing magnitude of reflected energy showed seasonal variations which are intimately connected with the changes in the surface features. The results showed that the magnitude of all kinds of vegetative soil cover depends on type of vegetation, its age and the soil present. The magnitude of reflectance from soil decreases with increasing amount of organic matter content and especially with moisture content because water greatly decreases reflectance from soil. Based on this information it can be seen that the computer-aided analysis of Landsat data can provide a great deal of information about soil moisture conditions.

Effects of Nutrient Regimes and Ectomycorrhizal Inoculation on Growth Parameters of Three Oak Species. R. W. DOUGLASS, Research Assistant and P. E. POPE, Assistant Professor, Department of Forestry and Natural Resources, Purdue University, West Lafayette, Indiana 47907._____Northern red (*Quercus rubra*), black (*Q. velutina*) and white oak (*Q. alba*) seedlings were grown in 10 cm X 10 cm X 26 cm containers under 16 hour photoperiod. Within species comparison of seedlings subjected to varying nutrient regimes (0, $\frac{1}{2}$ X, 1X, and 2X Hoagland's solution) and fungal inoculation rates (0 to 634 ml/ft³ *Pisolithus tinctorius*) indicated that stem height, leaf area, number of growth flushes, total dry weight and shoot to root ratio (S/R) increased with increasing amounts of available nutrients. Mycorrhizal development was slight and demonstrated no significant effect on the growth parameters measured. Among species, differences were significantly influenced by nutrient regime. Generally, black oak exhibited the greatest response to increased levels of available nutrients, followed in order by red and white oak. On the average, correlation between growth parameters differed among species. The within species correlations for

the aboveground components improved with increased nutrient status while the aboveground to root component was unaffected.

Intensive Culture Influences Growth and Nutrition of Containerized Black Walnut. J. M. BRAUN, Research Forester and P. E. POPE, Assistant Professor, Department of Forestry and Natural Resources, Purdue University, West Lafayette, Indiana 47907.——Successful production of high quality hardwood seedlings through the use of intensive culture and containerization has lagged far behind the accomplishments of coniferous nurseries using these techniques. However, the rewards of producing high quality seedlings *ie.* excellent survival after outplanting, and good early growth, continues to spur research towards this end. The objective of this study is to quantify the impact of a series of nutrient regimens on intensively cultured seedlings.

Pre-germinated black walnut (*Juglans nigra* L.) seeds were grown in 10 x 10 x 25 cm paper containers for a period of 13 weeks. Seedling density varied between 0.46 and 0.75/m² (5-8/ft²). Supplemental lighting provided a 16 hr. daylength. The nutrient treatments consisted of a distilled water control, a standard Hoagland's formulation and ¼x, ½x, and 2x the standard solution. Nutrient levels significantly affected stem length and stem, leaf, and total dry weight. The 2x Hoagland solution increased stem length and total dry weight by 50% and 45% respectively over the control. Foliar N, P, and K and food reserves in the stem and taproot were evaluated with regards to nutrient treatment and the physical characteristics of the seedlings.

Ambient-Temperature Storage-Not Good for Black Walnut Seed. ROBERT D. WILLIAMS, USDA Forest Service, North Central Forest Experimental Station, Bedford, Indiana 47421.——Black walnut (*Juglans nigra*) seed did not germinate after one full year in ambient temperature storage. Seedlots stored under roof but subject to outside temperatures, in Georgia, Tennessee, Illinois, Indiana, and Minnesota from January until April (seedlots were bagged in April and placed in cold storage until they were sown) and from January until October were fall sown at the Vallonia Nursery in Indiana. About 6% of the seed stored at outside temperature until April germinated but none of the seed stored at ambient temperature until October germinated.

Evaluation of the Suitability of Cooling Pond Water for Growth of Various Algae. RICHARD WHITMAN, Division of Science and Mathematics, Indiana State University, Evansville, Indiana 47701.——This study attempts to demonstrate the suitability of water parcels collected from the Cedar Bayou Generating Station's cooling lake for algae isolated from that study area. The algae were incubated at three different temperatures (25°, 29°, and 32° C) and in five different media representing four field water parcels collected plus an enriched nutrient medium which functioned as a control. In addition to the three different phytoplankton populations isolated from the field, a fourth laboratory grown algae served as the control algae.

The overall data suggests that caution should be exercised when laboratory reared species are used for test organisms. Differences in the algal growth potential may be related to the population's adaptive history and not to the quality of the test medium. Similarly, artificial media does not always demonstrate deficiencies in natural media and may, in fact, be less suitable for the existing population.

Translocation of Polychlorinated Biphenyls into Tomatoes from Contaminated Soil

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Introduction

In the ten years since the potential human health hazard of polychlorinated biphenyls (PCBs) was brought to public attention, the movement of PCBs through food webs has been studied extensively. In 1968 at least 1,291 Japanese were poisoned by rice oil contaminated with PCBs, twenty-two died (1). In 1966 PCBs were first measured in fish in Swedish waters (5). Since that time PCBs have been found in almost all waters tested. In some systems, especially the Great Lakes, many species of fish are unfit for human consumption due to the high concentrations of PCBs.

Although much research has been accomplished on measurement of PCBs in various components of ecosystems and on toxicology of PCBs in animal studies, there has been little published on PCBs translocated into crops grown for direct human consumption. Interest in this question heightened recently in central Indiana as organic farmers and backyard gardeners who used PCB contaminated sludge as a humus conditioner for their soils become concerned over the possibility of PCB accumulation into their crops.

Suzuki (10) has studied the translocation of PCBs into soy beans under greenhouse conditions and Iwata (4) into carrots under field conditions. Moza (7,8) has studied the metabolism of specific PCB isomers in a salt marsh grass (*Veronica beccabunga*) and in plant-water-soil systems. Sinclair (9) has shown the reduction of photosynthesis in the green algae *Chlorella* and Keil (6) has demonstrated the reduction of nucleic acid synthesis in marine diatoms.

PCBs are lipid soluble and have been shown to biomagnify through aquatic food webs (2). PCBs are stable to chemical and biological degradation and are estimated to have a half-life of over 30 years in the environment (3). Special incinerators at temperatures above 2,000° F are required to destroy PCBs. This extraordinary stability lead to the primary use of PCBs as an insulator in capacitors and transformers used throughout the electronics industry.

U.S. production of PCBs from its beginning in 1929 until it stopped in 1977 was 1,250 million pounds. Of this total only 55 million pounds have been incinerated. There are 440 million pounds distributed throughout the environment in the air, water and soil. There are still 750 million pounds in service that have the potential of entering the environment.

This investigation was designed to measure whether or not PCBs would accumulate in the fruit of the tomato when the plant was grown in PCB contaminated soil. The plants were grown from seed in the Butler University

greenhouse between February and June 1978. The extractions and gas chromatographic measurements were done at Argonne National Laboratory.

Methods

The control soil composition consisted of two parts potting soil to one part peat moss to one part sand. The contaminated soil composition consisted of three parts control soil to one part PCB contaminated sewage sludge taken from the Bloomington, Indiana sewage treatment facility.

The plants were staked and tied to keep them off the soil and spaced so as not to touch each other. As the fruit ripened it was harvested and placed into a Ball mason jar with an aluminum foil cap and frozen until extracted.

Single samples of the fruit, leaves, stems, roots and control soil were contaminated with a known quantity of Aroclor 1254 to determine the extraction efficiencies, all of which exceeded 97%.

The extraction of the soils and sludge was accomplished by placing a sample in an empty glass column (19 mm ID by 60 cm) with a glass wool plug and eluted with 20 volumes of 1:1 hexane/acetone. The extract was concentrated in a Kuderna-Danish concentrator.

The extraction of the plant samples was begun by homogenizing in 3:1 acetonitrile/water. The homogenate was then filtered and rinsed with hexane. The filtrate and rinses were diluted with three parts aqueous 1% sodium chloride solution and extracted with three portions hexane in a separatory funnel. The extract was concentrated in a Kuderna-Danish concentrator.

The plant, soil and sludge concentrates were then chromatographed on Florisil using 1% diethyl ether in hexane as the eluting solvent. After concentration, the elutant was analyzed for PCBs on a Perkin-Elmer model 3920-B gas chromatograph with an electron capture detector using a 6 foot by 2 mm ID glass column packed with 1.5% SP2250 and 1.95% SP2401 on 100/120 mesh Supelcoport.

The plants grown in the control soil (controls) were handled exactly the same way as the plants grown in the PCB contaminated soil (contaminated). A laboratory blank was a clean and empty glass beaker that followed the exact procedures as the control and contaminated samples.

Results and Discussion

The control plants were used to measure background PCB contamination in the greenhouse and the laboratory blank was used to measure PCB contamination from the analytical laboratory. The controls had a low PCB concentration as background ranging between two and 50 parts per billion, the blank had no detectable PCB concentration. The efficiency of the extraction techniques exceeded 97%. The reporting limits of the experimental samples are based on the concentrations of Aroclors extracted from the controls and the blank (refer to Table I).

The fruit, leaves, stems, roots and control soil all had concentrations of Aroclors below 0.05 parts per million (ppm). These levels are below the

reporting limits established by this study. Above these limits there appears to be no translocation of PCBs occurring into the fruit of the tomato.

TABLE I Concentrations of PCBs in parts per million in tomato samples

SAMPLE	AROCOR		
	1254	1248	1242
FRUIT	<0.01	<0.01	<0.01
LEAVES	<0.01	<0.01	<0.1
STEMS	<0.01	<0.005	<0.005
ROOTS	<0.05	<0.05	<0.05
SLUDGE	<5	<10	370 (± 150)
CONTAMINATED SOIL	<10	<10	170 (± 20)
CONTROL SOIL	0.2	<0.02	<0.02

Acknowledgement

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Leaf Size Variation at Cook's Woods

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Introduction

The relationship between leaf form and climate has been studied by ecologists (1, 2, 20, 25, 30) and paleobotanists (9, 30, 31, 32) for many years. Their studies have been concerned with two specific characters, average leaf size and leaf margin type. In 1915, Bailey and Sinnott (1, 2) postulated that leaf margin type and mean annual temperature were directly correlated. As temperature decreases, either with increasing altitude or decreasing latitude, the percentage of species having leaves with entire margins in a flora also decreases (1, 2). The only exceptions occur in physiologically dry areas, such as deserts, where the percentage of species having leaves with entire margins is always high, regardless of altitudinal or latitudinal position. Recent studies (31, 32, 33) using both modern and fossil floras have tended to substantiate these conclusions (1, 2). Raunkiaer (25) postulated that leaf size was directly correlated with mean annual precipitation. As mean annual precipitation decreases, the percentage of species having large leaves in a flora also decreases (25). Very little field work (9) has been carried out to validate this hypothesis.

Recently, ecologists have begun to mathematically model the relationship between leaf form and climate. For example, Givnish (20) postulates that optimal leaf size, as determined by the balance between transpiration, root costs, and photosynthesis, should be greatest in the tropics, decrease into the subtropics, increase into the temperate region, and finally decrease toward the poles. Although his (20) approach has provided significant new insights into the relationship between leaf form and climate, the results are only indirectly correlated with typical climatic variables such as temperature and precipitation. No distinction is made between altitudinal and latitudinal trends.

The majority of studies that have been carried out have attempted to correlate the leaf characteristics of broad regional floras with the general climate for the areas in which they live. Only recently have studies been undertaken attempting to relate leaf form and climate at the local level (16, 17, 18, 19). Most of these studies have been confined to the tropics (16, 17, 18). The results indicate that leaf margin type is not closely correlated with climate at the local level (16, 19). In contrast, leaf size is correlated with climate, at least to the extent that four foliar belts can be recognized in the tropical latitudinal region of the Western Hemisphere (14, 17, 18). Attempts to extend these foliar belts into the subtropical and warm temperate latitudinal regions have failed due to a lack of data (14). In this paper, the leaf size variation at Cook's Woods is analyzed and

compared with other data available from the warm temperate latitudinal region of the Western Hemisphere.

Materials and Methods

Cook's Woods, located north of the Mississinewa Reservoir in Miami County, Indiana (Sec. 34, T 27 N, R 5 E, Peoria Indiana Quadrangle) was chosen as the site for this study (Fig. 1). An analysis for stand disturbance (26) indicated that Cook's Woods is a secondary successional forest. Two parallel line transects were run along the ridge top at the site. Each transect was 400 m long. Importance values for the dominant species in the canopy and understory were determined using the point-centered quarter method (11, 12, 24). The sampling points were located at 20 m intervals along the transects. The

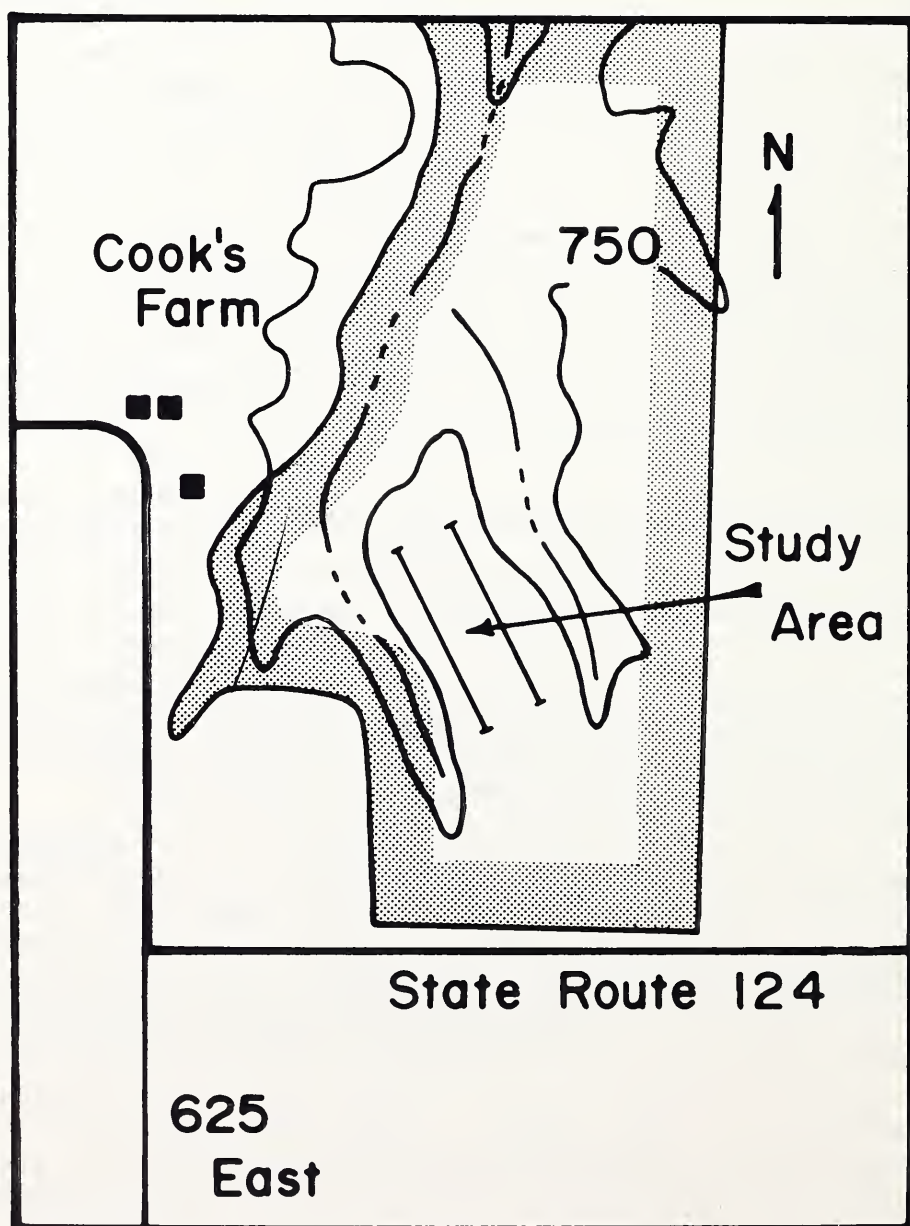


FIGURE 1. Location of the study area in Cook's Woods.

dominant species in the shrub layer were studied using 10 quadrants located at 20 m intervals. Each quadrant was 100 m square. The importance value for each species encountered (Table I) was calculated using the method of Curtis and McIntosh (5, 6).

TABLE I Importance values and average leaf areas of the woody, dicotyledonous trees, shrubs, and vines found at Cook's Woods. The species were identified using Deam (7, 8).

Species	Importance Value (Canopy and Understory)	Importance Value (Shrub Layer)	Leaf Size
<i>Carya glabra</i>	116.3		30.6
<i>Acer saccharum</i>	33.1	59.8	63.5
<i>Ostrya virginiana</i>	26.5	60.7	19.3
<i>Carpinus caroliniana</i>	25.2		21.8
<i>Carya ovata</i>	24.2		34.7
<i>Quercus alba</i>	21.8	21.4	69.9
<i>Fagus grandifolia</i>	10.1	10.3	35.7
<i>Liriodendron tulipifera</i>	9.0	1.5	124.8
<i>Quercus rubra</i>	8.7		58.9
<i>Prunus serotina</i>	7.8	20.0	22.7
<i>Crataegus punctata</i>	5.4	22.3	10.5
<i>Populus grandidentata</i>	2.5		36.3
<i>Ulmus rubra</i>	2.5	38.7	51.2
<i>Quercus mühlenbergii</i>	2.0		70.9
<i>Morus rubra</i>	1.8	9.0	89.4
<i>Cornus florida</i>	1.6	3.7	35.1
<i>Celtis occidentalis</i>	1.6		40.5
<i>Juniperus virginiana</i>		1.2	
<i>Viburnum acerifolium</i>		2.7	39.8
<i>Asimina triloba</i>		3.3	118.0
<i>Cornus alternifolia</i>		2.7	24.0
<i>Fraxinus americana</i>		16.9	30.2
<i>Diervilla lonicera</i>		6.4	10.7
<i>Lindera benzoin</i>		16.8	37.1
<i>Ribes cynosbati</i>		2.5	12.0
<i>Rubus occidentalis</i>			19.7
<i>Sambucus canadensis</i>			21.8
<i>Gleditsia triacanthos</i>			2.0
<i>Euonymus atropurpureus</i>			30.9
<i>Rubus flagellaris</i>			24.4
<i>Platanus occidentalis</i>			144.2
<i>Parthenocissus quinquefolia</i>			36.8
<i>Vitis sp.</i>			74.1
<i>Carya cordiformis</i>			59.4
<i>Tilia americana</i>			91.7

The average leaf area was calculated for every species of woody, dicotyledonous tree, shrub, and vine encountered along the line transects as well as for several additional species encountered while walking through the woods. The area of 50 leaves from each species encountered was determined using a dot planimeter (13, 21). The average leaf area for each species (Table I) was calculated using a modified version of a computer program provided by Sokal and Rohlf (27).

To facilitate the comparison of the results obtained in this study with those

from other localities both in the temperate zone (3, 10, 30) and in the tropics (14, 17, 18), the percentage of species having large leaves (greater than 20.25 sq cm in area, *sensu* 9) at each locality was plotted on a modified version of Holdridge's (22, 23) life zone chart (Fig. 2). The chart was modified to show only the warm temperate life zones from which data were available. Each locality was plotted on the life zone chart using climate data from the U.S. Department of Commerce Environmental Data Service (28, 29).

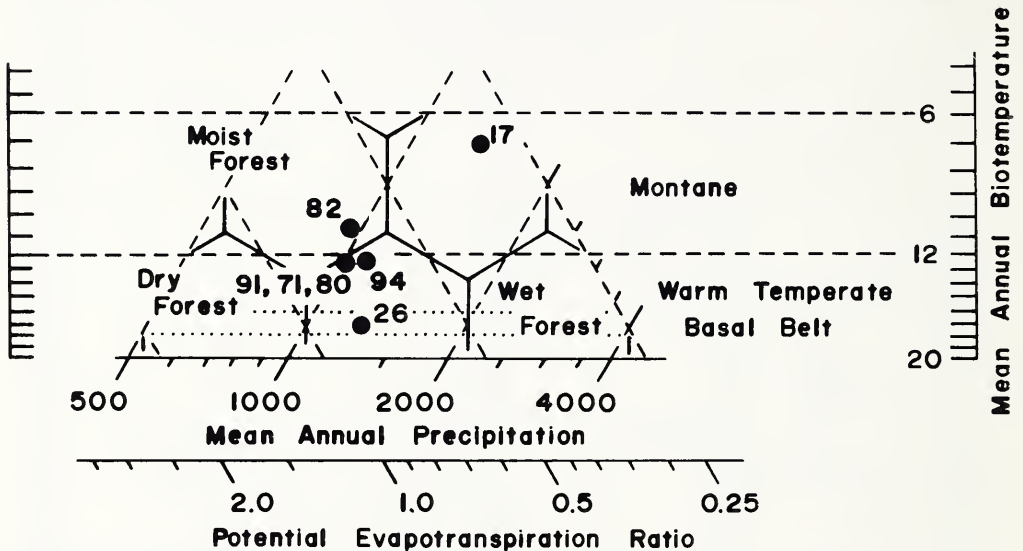


FIGURE 2. Variation in leaf size at the seven sample sites that have been studied in the Western Hemisphere (see text for details of chart construction).

Results and Discussion

The species with the highest importance value at Cook's Woods is *Carya glabra* (I.V. = 116.3, Table I). No other species in the canopy and understory had an importance value approaching that of *C. glabra*. The shrub layer is dominated by seedlings of *Acer saccharum*, *Ulmus rubra*, *Ostrya*, and *Carpinus* (Table I). The lack of any clear dominants at Cook's Woods is an indication of the secondary nature of the forest. Based on comparisons carried out between virgin and secondary tropical forests (15), it is doubtful that the successional nature of Cook's Woods would affect the results of this study. The analysis of leaf size variation is dependent on the species present and not the structure of the mature vegetation (15).

The percentage of species having large leaves (*sensu* 9) at Cook's Woods was 82.4%. This percentage compares favorably with the percentages found at other localities in the lowland temperate forests of North America. One other site (Fig. 2) has been studied in Indiana (10, 13). The percentage of species having large leaves at Cedar Bluffs, Indiana, was 94.4% (10, 13). Three localities (Fig. 2) were analyzed in the Cincinnati area (30). The percentages of species having large leaves at these sites were 90.9% (mesophytic climax forest), 71.1% (upland forest), and 80.0% (grassland).

All of these values compare favorably with the average percentage of species having large leaves in the first foliar belt of the tropical lowlands of the

Western Hemisphere (83.0%, 14). The high percentage of species having large leaves in warm temperate forests as well as the correspondence in leaf size between these forests and those of the tropics were pointed out by Givnish (20). These conclusions are significantly different from those reached by Raunkiaer (25). However, complete agreement with the theory of Givnish (20) was not found. In the Smoky Mountains, the percentage of species having large leaves decreases with increasing altitude. Only 16.7% of the species in the heath balds on Mt. LeConte had large leaves (3). At one warm temperate locality in southern Brazil (the temperate gallery forest at Horto Botânico), the percentage of species having large leaves was 25.7% (4). These percentages agree with the predictions of Raunkiaer (25) and not of Givnish (20). Based on the data available, there is insufficient information to choose between these two theories (20, 25). Further field research will have to be carried out before a definite correlation between leaf size and climate can be proven.

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Evaluation of Heterosis in Wheat

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Introduction

Currently there is much interest in developing commercial F_1 hybrid wheats. Hybrids have great potential for increasing the world's future food supply. The genetic mechanism for hybrid wheat is a cytoplasmic male sterile (CMS)—genetic fertility restorer system similar to the one widely used in sorghum. The cytoplasm of the A-line (female) currently used in commercial hybrid production is derived from the wheat relative *Triticum timopheevi*. The R-line (male) pollinator has the genetic nuclear restorer factors. The F_1 hybrid is fertile and is used for the commercial crop.

In breeding new A-lines and R-lines an early test for potential hybrid vigor in the F_1 generation is desirable. A-lines may be produced relatively easily by crossing an A-line (CMS) as female to a normal line (termed B-line) and backcrossing to the B-line several times to develop the new A-line with the agronomic type of the B-line. Developing R-lines has been much more complex and very few satisfactory ones have been produced. An early evaluation system for detection of exceptional levels of heterosis in wheat is needed so that efforts can be maximized in the development of A-lines and R-lines in germplasms which combine well in hybrids.

Early studies of hybrid vigor in wheat were conducted with handcrossed seeds and reduced seeding rates either by space-planting individual seeds or by planting in hill plots (2, 4, 7). The question of whether or not hybrid vigor under reduced seeding rates were greater than under seeding rates normal for the commercial crop still remains. Comparisons between hill plots and nursery yield plots have been made using the more plentiful F_2 generation seed (4). Unlike the case for corn, hybrid vigor in wheat in the F_2 generation is much less than 50% of that of the F_1 generation. Therefore, F_2 generation tests are not considered useful. Grain yield of hybrids has been subdivided and studied as individual components. Overdominance and heterosis is common for spike number and total yield. Additive gene action and partial dominance are more commonly observed for number of seeds per spike and weight per seed (2, 8). At reduced seeding rates and favorable environmental conditions one might expect greater heterosis for spike number and grain yield than under seeding rates normal for the commercial crop.

Chemical male gametocides hold excellent promise for the development of hybrids to evaluate hybrid vigor. Those chemicals now available commercially either are not completely effective as male gametocides or have a deleterious growth regulator effect carryover in the hybrid.

Lines termed "partial restorers" frequently occur in the development of R-lines (8). These have CMS cytoplasm and are fertile in the homozygous state.

The F_1 obtained from crosses of these partial restorers with A-lines are partially fertile. Crosses can be made in isolation using a partial restorer as the pollen parent and several A-lines as the female seed parents and utilizing wind-blown pollen (6, 8). Pollen of wheat is transported relatively short distances in a volume adequate for good seed set of A-lines (1).

Our experiment was designed to compare the expression of heterosis in F_1 wheat hybrids in hill plots at a reduced seeding rate with heterosis in nursery yield plots at a normal seeding rate. Heterosis, used in this contest, is that increase above the average of the two parents.

Materials and Methods

The amount of heterosis in wheat from hill plot measurements was compared to that from nursery yield plots for grain yield and kernel weight. F_1 hybrid seeds were obtained by crossing CMS A-lines with restored R-lines. The parents and crosses are show in Table I. The R-lines are of the Monon cultivar type with nuclear restorer factors from *T. timopheevi* and CMS cytoplasm from *T. timopheevi*, as described previously (7). The A-lines have CMS cytoplasm from *T. timopheevi*.

TABLE I *Parents and hybrid number designation.*

Female parent	Male parent		
	68846L1	68846L2	68846L3
CMS Monon	1	4	7
CMS 5724	2	5	8
CMS Knox 62	3	6	9

The hybrids and parents were planted in hills 30.5 cm apart at a seeding rate of 15 seeds per hill. One hill was considered as a plot. The hybrids and parents were planted also in nursery yield plots adjacent to the hill plot experiment. The nursery plot consisted of four rows, 2.44 m long and 30.5 cm apart, with the center two rows harvested for yield. Seeding rate was 25 seeds per 30.5 cm². A split-plot design was used in each type of planting with hybrid combinations as whole plots and parents and the F_1 generation as subplots. Eight to 40 replications were used in hill plots and one to four in nursery plots, as permitted by seed supplies. Within the subplot two-thirds of the plants (parents) were fertile and one third, F_1 's, was partially fertile. Kernel weight was determined by weighing 200 randomly selected kernels. Pollen to fertilize the partial male-sterile F_1 's was supplied by male fertile plants and fertile flowers in the experiment and from a wheat field adjacent on the west.

Analyses of covariance for the two experiments were used due to incomplete fertility restoration in the F_1 hybrid. Seed set (percent) was determined for each hybrid and for their corresponding parents, thus allowing adjustment of plot yields for incomplete seed set. For the hybrids, the means for seed set ranged from 60-70%, whereas means for parents ranged from 86-93%. The equation for an adjusted treatment is given by the following model:

$$\text{Adj } \hat{Y} = Y_{ijk} - b(X_{ijk} - \bar{X} \dots)$$

The error regression coefficient is b with X and $\bar{\chi}$ as the covariate and mean of the covariates, respectively.

A fully least squares analysis generated a design matrix by computer programming using 0's and 1's. Simple linear regression analyses with all observations (Y) and all X 's were calculated. The "extra sum of squares" principle (3) was used on the adjusted values (5). The sequential F-test (3) was used to obtain the error regression coefficient. A harmonic mean square was used to obtain the error mean square for the analysis of variance of the combined planting technique.

Results and Discussion

Yield and kernel weight comparison for hybrids, R-lines, and fertile female parent counterparts of A-lines are presented for three hybrids (Table II). The yields in nursery plots were considerably higher than those in hill plots where seeding rates were reduced. The precision of measurement of differences in yields was higher also in the nursery plots as shown by LSD estimates. Differences between nursery plots and hill plots for kernel weights were small. Kernel weight was measured with somewhat greater precision in nursery plots.

TABLE II Means of three hybrids and their parents for yield (adjusted for fertility) and for kernel weight.

Genotype	Yield (bu/ A)		Kernel weight (g)	
	Hill plot	Nursery plot	Hill plot	Nursery plot
Hybrid 1	32.7	45.0	6.16	6.32
Monon	31.5	47.9	6.47	6.31
68846L1	33.4	44.5	6.37	6.26
LSD*	5.6	2.1	0.21	0.11
Hybrid 2	35.3	42.8	6.68	6.79
Purdue 5724	25.5	49.5	6.74	6.70
68846L1	34.7	49.1	6.31	6.27
LSD*	5.9	2.2	0.21	0.11
Hybrid 3	33.2	40.1	6.53	7.06
Knox 62	31.8	49.0	7.20	6.82
68846L1	33.0	49.3	6.36	6.38
LSD*	10.5	3.8	0.38	0.20

*LSD at .05 level of significance.

For the nursery experiment highly significant differences among hybrids were found for grain yield and for kernel weight. Three of 9 hybrids were significantly different from their parents for yield and 7 of 9 for kernel weight (Table III). Yields of the parents ranges from 40.4 to 50.0 bu/ A. Heterosis above the mid-parent value was not significant for adjusted yields or kernel weight for any hybrid. Most hybrids exceeded the lower yielding parent but not the higher yielding parent.

The absence of heterosis was unexpected although the parents were somewhat related. Patterson and Bitzer (9) observed significant heterosis expressed in hybrids tested with the greatest amounts produced in hybrids of

TABLE III *F values from analysis of variance for yield and kernel weight in the nursery row experiment.*

Source	df	F. values	
		Yield	Kernel weight
Hybrids (X)	9	23.24**	22.47**
S.E.	18	5.06	0.13
Parents/ hybrid 1	2	2.04	0.06
2	2	1.73	5.95**
3	2	1.20	3.91*
4	2	7.11**	1.24
5	2	4.21*	4.29*
6	2	0.52	10.83**
7	2	1.45	4.52*
8	2	3.58**	11.78**
9	2	1.38	3.33*
S.E.	35	3.25	0.17

*,**Significant at the .05 and .01 levels, respectively.

unrelated parents. Heterosis for yields may be expressed better in more favorably environments. Heterosis was not expressed for kernel weights where hybrids had fewer kernels per spike due to sterile flowers. Kernels formed from wind-blown pollen tend to be shriveled, perhaps from delayed fertilization of the flowers (our observation and hypothesis). Kernel weight has not been shown generally to exhibit heterosis (2, 8).

In the hill plot experiment, yields ranged from 22.8 to 40.1 bu/A for the parents. Differences among hybrids were significant for kernel weight but not for yield. Differences between parents and hybrids were significant for yield in 7 of 9 comparisons and in all 9 comparisons for kernel weight (Table IV). However, heterosis was not significant for grain yield or kernel weight.

TABLE IV *F values from analysis of variance for yield and kernel weight in the hill plot experiment.*

Source	df	F. values	
		Yield	Kernel weight
Hybrids (X)	8	1.73	6.94**
S.E.	182	7.53	.41
Parents/hybrid 1	2	0.29	5.81**
2	2	8.33**	11.24**
3	2	0.05	13.16**
4	2	3.39*	3.27*
5	2	8.18**	14.36**
6	2	4.92**	28.49**
7	2	7.36**	6.82**
8	2	10.18**	15.52**
9	2	20.23**	27.67**
S.E.	340	8.87	0.32

*,**Significant at the .05 and .01 levels, respectively.

The analysis of variance for the two plot techniques revealed significant interactions in grain yields for plot type x hybrids; parents x hybrids; and plot type x parents x hybrids (Table V). No significant interaction occurred for

TABLE V *F* values from the analysis of variance for yield and kernel weight with combined plot techniques.

Source	df	F values	
		Yield	Kernel weight
Plot types (K)	1		
Hybrids (H)	8	1.77	1.37
K x H	8	2.11*	0.08
Parents P x H	18	7.08**	1.30
K x P x H	18	6.48**	0.30
S.E.		2.16	0.32

*,**Significant at the .05 to .01 levels, respectively.

kernel weights. In general the hybrids expressed a lower magnitude of difference from their parents in the nursery plots. The precision of measure of differences was better in the nursery row plots. The individual LSD's for comparing hybrids with parents for yields ranged from 2.0 to 3.8 and averaged 2.4 whereas LSD's for the hill plots ranged from 5.5 to 10.5 and averaged 6.4.

Our experiment failed to detect the hybrid vigor expected. Three probable reasons for this failure are somewhat related parents, a below average environment, and incomplete seed set. The experiment could be improved by providing a high pollen load using a mixture of cultivars in alternate strips of pollen source to yield test areas in about 1:1 ratio of areas (eg. 2.44 m strips) (1). We believe that partially restored R-lines can be utilized in a systematic search for germplasm combinations which express exceptional levels of hybrid vigor.

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Black Walnut Growth Increased When Interplanted With Nitrogen-Fixing Shrubs and Trees

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Introduction

Because black walnut (*Juglans nigra* L.) trees are valuable for both wood and nuts, new ways are always being sought to accelerate their growth. Recently, it was found that walnuts grow more rapidly when interplanted with nitrogen-fixing plants. We conducted this study to determine which species of nitrogen-fixing plant has the greatest affect on walnut growth.

Methods

In the spring of 1967, 1-year-old nursery-grown walnut seedlings were planted at an 11- by 11-foot spacing in southern Indiana on a well-drained Parke silt loam soil with a 2 to 6% slope.¹ Two years later, autumn olive (*Elaeagnus umbellata* Thumb.), European alder (*Alnus glutinosa* (L.) Gaertn.), and black locust (*Robinia pseudoacacia* L.) were interplanted with the walnuts and lespedeza clover (*Lespedeza striata*) was broadcast sowed with the walnut seedlings. The nurse trees were planted between the walnut trees, which altered the spacing to 11- by 5½-feet after interplanting. Each treatment plus a pure walnut control was randomly assigned in each of three replications. The interplanted plots contained six rows of six walnut seedlings, five rows of six nurse trees, and a border row of walnut and nurse trees surrounding the 36 walnuts being studied.

Weeds were controlled for 5 years with amazine at a rate of 4 pounds active ingredient per acre in all plots except lespedeza clover. An 18-inch strip of the herbicide was sprayed between the rows each spring. Alder and black locust were especially susceptible to the herbicide and autumn olive was somewhat susceptible. All dead interplanted trees were replanted after the first two growing seasons. Herbicide was not used in the lespedeza plots, and the clover was never established, even after resowing.

After the fifth growing season some of the locust trees were overtopping the walnut trees so all locusts were killed by frill-girdling. The walnut trees were correctively pruned as needed (2). Height and diameter of the study trees have been measured annually and difference verified by analyses of variance.

Results

Height differences among treatments were apparent after the second growing season (Fig. 1). Walnut trees interplanted with European alder were tallest after the second and third growing seasons and differences among

¹ We thank Bob Burke and the Pierson-Hollowell corporation for use of their land and continuing cooperation.

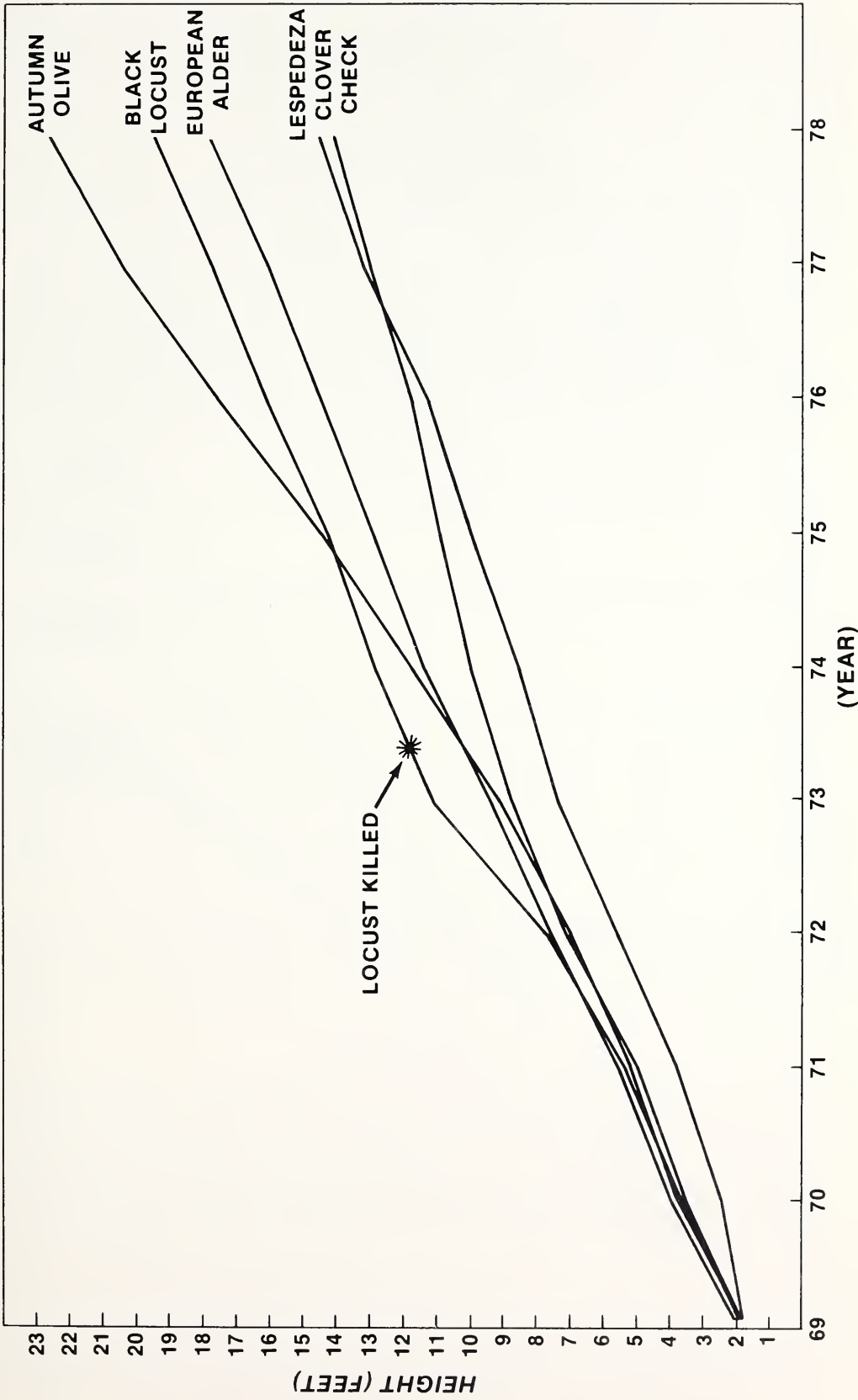


FIGURE 1. Height growth of black walnut interplanted with four nitrogen-fixing species.

treatments were statistically highly significant. Height differences of the walnut among treatments were not statistically significant after the fourth growing season but trees interplanted with black locust were tallest. The height advantage of walnut interplanted with locust continued through the fifth and sixth growing season and differences among treatments were significant, statistically. Walnut interplanted with autumn olive became tallest after the seventh growing season and have remained tallest through the tenth growing season. Differences among treatments have been statistically highly significant. After the 1978 growing season, 10 years after interplanting, walnut interplanted with autumn olive was 3.1 feet taller than walnut interplanted with locust, the second best nurse species, and 8.4 feet taller than walnut in the check plots.

Diameter differences among treatments were not statistically significant until after the eighth growing season (Fig. 2). Walnut interplanted with autumn

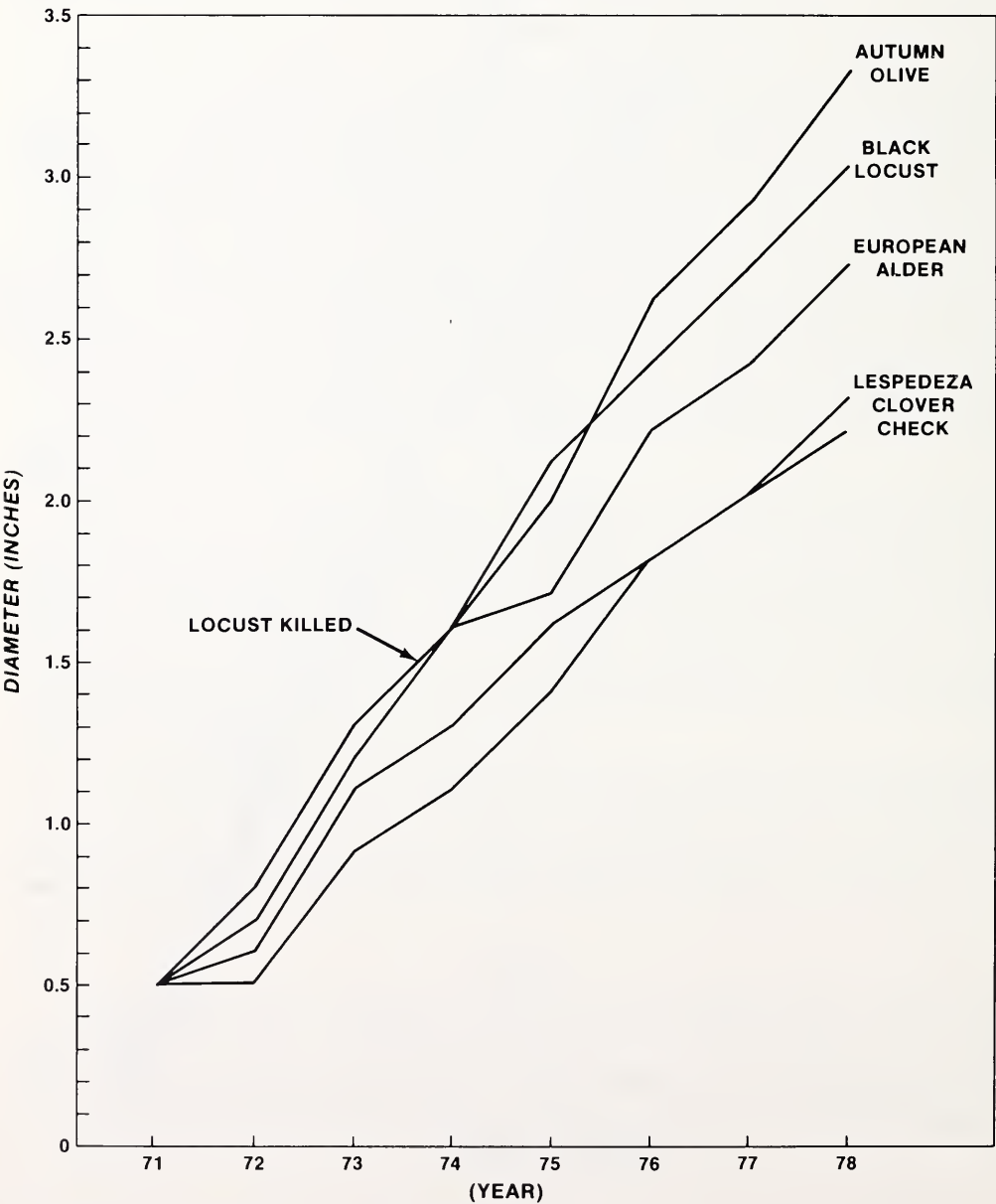


FIGURE 2. Diameter growth of black walnut interplanted with four nitrogen-fixing species.

olive had the largest diameters after the eighth season and continued to have the largest diameters through the tenth growing season. After the tenth season, diameter of the walnut interplanted with autumn olive was 0.3 inch larger than those interplanted with locust and 1.1 inch larger than trees in the check plots.

Discussion and Conclusions

Foliage of the walnut trees interplanted with autumn olive, black locust, and European alder was noticeably greener during the 1978 growing season than walnut foliage in the check plots. The lush green color may be an indicator of greater nitrogen content, but foliage samples have not been analyzed for mineral content. The walnuts in the check plots are not as straight and have required more pruning than those in the other plots. Therefore, the nurse trees must have helped the walnut trees grow straighter and aided in naturally pruning them.

Besides increasing walnut growth, the nurse trees provide economically and ecologically beneficial products. Autumn olive, with its dense shrubby habit and bountiful berry crop, provides cover and food for several wildlife species and pulpwood and fence posts can be obtained from European alder and black locust, respectively.

Herbicides were not used in the lespedeza clover plots and through the eighth year the competition resulted in an even slower growth rate for the walnut than the check plots.

Results from this study show that autumn olive is an excellent nurse species to interplant with black walnut. Walnut interplanted with autumn olive was tallest after the seventh growing season and increased in superiority through the next three growing seasons. European alder and black locust also increase height and diameter growth of the walnut over pure walnut, but walnut interplanted with autumn olive grew fastest. Funk *et al.* (3) reported similar growth increases for interplanted walnut in Missouri and Illinois plantations.

Autumn olive's only possible drawback is that it is widely spread by the birds and other wildlife that use the berries for food (1). Although its invasion to surrounding areas detracts from its desirability as a nurse tree, unwanted autumn olive may be controlled by regular mowing.

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CELL BIOLOGY

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Ultrastructure of the Anal Organ of the Larva of *Drosophila melanogaster*. MOHINDER S. JARIAL, Department of Physiology and Health Science, Ball State University, Muncie, Indiana 47306._____The anal organ of full-grown larva of *Drosophila melanogaster* (Oregon R, wild type) consists of two symmetrical plates located ventrolaterally on either side of the anus. Each plate consists of a thin cuticle which is closely applied to the underlying single layer of large, cuboidal, epithelial cells. The apical (external) plasma membrane of the epithelial cells is highly infolded and the infoldings are covered by a particulate coat on the cytoplasmic surface. The mitochondria lie adjacent to the apical region and small ones occasionally penetrate between the infoldings. On the basal surface (facing the haemocoel) the plasma membrane is also infolded with large mitochondria in close association to the membranes. A thin basement membrane separates the epithelial cells from the haemocoel. The lateral borders of the cells are bridged by septate desmosomes. The cells are rich in mitochondria and contain rough endoplasmic reticulum and numerous multivesicular bodies. Tracheoles ramify the cells and penetrate deeply into the basal region.

When larvae are treated briefly with 1% silver nitrate solution, the anal organ becomes darkly stained and the silver grains after passing through the cuticle reach the apical regions of the epithelial cells.

The observed ultrastructural features of the anal organ are characteristic of transporting epithelia and are consistent with an osmoregulatory function.

Role of a Calcium Binding Ribonucleoprotein in the Ontogenesis of Electrical Activity in the Developing Chick Embryo Brain. Z. IQBAL and N. C. SHARMA, Indiana University School of Medicine, Indianapolis, Indiana 46202._____Electrical activity in the developing chick embryo brain has been found to appear around the 4th day and matures by 12th day of incubation. Our laboratory has been engaged in the evaluation of some key cellular components (Iqbal and Talwar, J. Neurochem. **18**:1261, 1971; Talwar and Iqbal *In* Macromolecules and Behavior, ed. J. Gaito, 1972) involved in the manifestation of this functional characteristic in the developing chick embryo brain. In this report, we present the data concerning an organ specific calcium binding ribonucleoprotein (NP) in relation to the development of electrical activity in growing chick embryo brain. NP purified from goat brain according to the method of Sharma (J. Sci. Industr. Res. **33**:662, 1974) was used as a marker and the brain extracts from chick embryos of various ages were analyzed by

electrophoresis on 7.5% acrylamide gels. The protein in the chick embryo brain identical to NP as regards to its molecular weight and immunological properties appeared around the 6th day of incubation and increased in quantity with age of the embryo reaching a plateau on day 12. A parallelism between the onset and maturation of the electrical activity and the development of NP suggests that this protein is involved in the manifestation of the electrical activity in the developing chick embryo brain, probably by regulating the concentration of Ca^{2+} in the tissue.

Toxic Effects of the Vinca Alkaloids on Cultured Rat Midbrain: Correlation with Clinical Neurotoxicity. KATHLEEN L. KING and GEORGE B. BODER, Department of Cell Biology, Lilly Research Laboratories, Indianapolis, Indiana 46206._____The three Vinca alkaloids currently in use as cancer chemotherapeutic agents differ in their clinical neurotoxic potential. Neurotoxicity is the dose limiting factor for Vincristine, but it is a less serious problem with Vinblastine. Vindesine appears to be intermediate. These effects may reflect pharmacokinetics and microtubular binding in nervous tissue and tumor cells. A system suitable for studying the factors underlying clinical differences and predictive of neurotoxicity would augment the development of novel non-neurotoxic Vinca molecules. Rodent animal models are inadequate for the study of this neurotoxicity because Vincristine produces a severe myopathy rather than neuropathy. Differences in pharmacokinetics can be avoided by studying the direct effects of the Vinca alkaloids on primary cultures of neuronal and glial cells. Vincristine at a dose as low as $0.004 \mu\text{g}/\text{ml}$ affects the cells with processes in cultures of dissociated newborn rat midbrain. In 3 day old cultures after 24 h of drug treatment, there is a loss of processes and swelling of the cell body. We have used this observation as the basis for a quantitative assay of the toxicity of a series of Vinca compounds, and have found that for a dose range of 0.1 to $0.004 \mu\text{g}/\text{ml}$ the relative toxicity of Vincristine, Vinblastine and Vindesine in this system correlates with their relative clinical neurotoxicity. Validation of the predictive elements of this system awaits clinical experience with novel Vinca compounds.

The Effect of Endurance Training on the Ultrastructure of Diabetic Skeletal Muscle. LARRY R. GANION and DAVID COSTILL, Ball State University, Muncie, Indiana 47306._____To study the effect of endurance training on the morphology of the skeletal muscle fiber of the diabetic, muscle samples were obtained by needle biopsy from the gastrocnemius of subjects at the commencement and completion of a ten week training program and prepared for electron microscopy. The participants ran five days per week at a stress level of 75% of their maximum work load. The muscle samples were immersed in 3% glutaraldehyde for four hours, rinsed in 0.1 M Sorenson's phosphate buffer (pH 7.2), postfixed for one hour in 1% osmium tetroxide, and embedded in Epon 812. Ultra-thin sections were cut on a Porter-Blum MT-2B ultramicrotome, stained with uranyl acetate and lead citrate, and viewed with an RCA EMU-3C electron microscope. Endurance training does alter the fine structure of the diabetic skeletal myofiber. These alterations include: a decrease in glycogen particles, a definite increase in lipid vacuoles, and mitochondrial swelling. Dense

bodies are frequently seen at the periphery of the untrained diabetic myofiber. These pleomorphic structures, composed of aggregates of electron dense particles and lipid droplets, appear to decrease in number as a result of training. These morphological observations support the conclusion that training effects the physiology of the diabetic myofiber, particularly lipid metabolism. The significance of the dense body reduction in trained muscle is not known and merits further study.

Effects of Cytochalasins on Growing Fungal Hyphae. J. A. SWEIGARD, B. L. HOLAWAY and S. N. GROVE, Goshen College, Goshen, Indiana 46526.——Cytochalasin B(CB) at 50-100 $\mu\text{g/ml}$ inhibits germination and hyphal growth in *Gilbertella persicaria*. When sporangiospores are germinated in the presence of CB the initial spherical growth (swelling) stage occurs as expected but the orderly initiation of germ tubes (polar growth stage) and continued hyphal growth does not occur. The germlings continue to enlarge (swell) and become giant cells sometimes with bulbous outgrowths instead of germ tubes. If CB is introduced after germ tube initiation subsequent growth results in knob-like swellings at the tips of existing structures. Our light and electron microscopic observations along with evidence from the literature may lead to a clarification of parts of the current model for hyphal tip growth. The model proposes that secretory vesicles produced in the subapical regions migrate to the apex and deposit the necessary materials for polarized growth but until now we have been unable to explain the mechanisms of this migration. If cytochalasins are active against actin microfibrils in fungal hyphae then any actin-dependent vesicle migrations would cease in the treated germlings. The result would be a non-polarized, spherical type of growth of the type we have observed. Therefore, we postulate that the vesicle transporting mechanism is actin dependent in these tip growing fungal hyphae.

Cytidine 5'-Monophosphosialic Acid Synthetase from Rat Liver. KIM E. CREEK, DIANE WEISMAN and D. JAMES MORRÉ, Department of Biological Sciences and Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, Indiana 47907.——The incorporation of sialic acid into sialic acid-containing polymers, such as glycoproteins and glycolipids, requires CMP-sialic acid as the sialic acid donor. Cytidine 5'-monophosphosialic acid synthetase catalyzes the synthesis of CMP-N-acetylneuraminic acid (CMP-NAN) and inorganic pyrophosphate from cytidine triphosphate (CTP) and N-acetylneuraminic acid (NAN or sialic acid). Two assay methods were used to monitor the activity of CMP—NAN synthetase in isolated rat liver organelle fractions. A modified method of the thiobarbituric acid assay of Warren (J. Biol. Chem. 234, 1971, 1959) allowed the determination of CMP-NAN in the presence of large quantities of unreacted substrate. A second assay procedure used ^{14}C -NAN and separation of products and substrate by descending paper chromatography. Kinetic properties of CMP-NAN synthetase determined by either or both procedures may provide clues to the mechanisms that regulate the levels of sugar nucleotide within the cell and further our understanding of the processes involved in the biosynthesis of sialic acid-containing heteropolymers. Supported in part from a grant from the NIH CA 18801.

Does Cytidine 5'-Monophosphosialic Acid Synthetase Exist in Golgi Apparatus Isolated from Rat Liver? K. E. CREEK, D. WEISMAN and D. J. MORRÉ, Department of Biological Sciences and Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, Indiana 47907.——Cytidine 5'-monophosphosialic acid (CMP-NAN) synthetase is widely distributed in animal tissues. A report by Kean (J. Biol. Chem, 245, 2301, 1970) has shown that the predominant subcellular location of the enzyme is the nucleus. If the sole location of the enzyme is the nucleus, a problem of cellular logistics arises since the predominant cellular location of the sialyltransferases of CMP-NAN utilization is the Golgi apparatus. An investigation was conducted to determine if Golgi apparatus isolated from rat liver exhibited significant CMP-NAN synthetase activity. Golgi apparatus incubated in the presence of 5 mM NAN and 5 mM CTP synthesized CMP-NAN at a rate of 45 nmoles/hr/mg protein. This activity apparently is not due to contamination of the isolated Golgi apparatus by cellular organelles other than nuclei. Studies are in progress to determine by several independent procedures more precisely the levels of potential contamination of the Golgi apparatus fractions by nuclear material. Supported in part by a grant from the NIH CA 18801.

Histological and Growth Characteristics of Transplantable Hepatomas and Squamous Cell Carcinomas Induced in the Rat by the Carcinogen N-2-Fluorenylacetamide. T. M. KLOPPPEL, L. B. JACOBSEN, D. M. MORRÉ, P. FINK and D. J. MORRÉ, Departments of Biological Sciences and Foods and Nutrition and Purdue Cancer Center, Purdue University, West Lafayette, Indiana 47907.——Hepatocellular carcinomas and squamous cell carcinomas were induced by oral administration of the carcinogen N-2-fluorenylacetamide. Tumors arose 6 to 10 months following carcinogen ingestion when fed at a level of 0.025% for 16 weeks. Tumors were removed aseptically and transplanted subcutaneously to a syngeneic recipient or processed for tissue culture. The cytological and histological observations were contrasted and compared with growth rates and the ability to metastasize. The squamous cell carcinomas appeared as highly differentiated, fast-growing, keratinizing and non-invasive tissues which were readily cultured and transplanted back into the animal. The hepatomas were more difficult to grow in tissue culture and to transplant back into the animal. Hepatomas varied from poorly to highly differentiated and some showed metastasis to the lungs. Growth rates were variable and corresponded somewhat to degree of anaplasia. Work supported in part by a grant from the NIH CA 18801 and an Institutional Grant from the American Cancer Society.

The Effect of Electrically Induced Hyperthermia on Transplantable Tumors in the Rat. J. PEARCE, T. M. KLOPPPEL, D. GOETZ, K. E. CREEK, J. WALTER, L. A. GEDDES, M. J. FREEMAN and D. J. MORRÉ, Biomedical Engineering Center, Institute of Interdisciplinary Engineering Studies and Purdue Cancer Center, Purdue University, West Lafayette, Indiana 47907.——Subcutaneous-transplantable hepatomas and squamous cell carcinomas originally induced in the rat by feeding N-2-fluorenylacetamide were treated with radio frequency electrical current at 500 KHz to induce hyperthermia. A typical current of 100 mA was required to establish a tumor temperature of 42 to 44 C. Electrical

contact was by means of gelled deformable electrodes. Temperature was monitored by an electrode contact thermistor, a subcutaneous thermocouple, and a calibrated thermograph. For each treatment, a temperature between 42 and 44.5 C was reached within 4 min. and was maintained for 11 min. Animals received 1, 2 or 3 treatments at 2 day intervals. Growth rates of various treated and untreated tumors were compared. The transplantable squamous cell carcinoma appeared to be more sensitive to heat than were the hepatomas. In some animals the treatments were tumoristatic, while there was complete remission in a few animals. Histological examination revealed necrotic areas in treated tissue with neutrophilic infiltration. The size of tumor mass at the time of initiation of the treatments seemed to influence the overall success. Smaller tumors responded most consistently to treatment. Supported in part by grants from the Indiana Elks and an Institutional Grant from the American Cancer Society.

The Distribution of Anionic Sites on Brush Border Membranes of Neonatal Rat Intestinal Absorptive Cells. RALPH A. JERSILD, JR., Department of Anatomy, Indiana University Medical Center, Indianapolis, Indiana 46202._____The distribution and behavior of anionic sites on the microvillous surface of newborn rat jejunal absorptive cells were studied using polycationic ferritin (PCF) as a visual probe, and compared with anionic sites previously described for adult intestine. Intestinal segments from 1 to 22 day old rats were incubated in PCF prior to fixation and preparation for electron microscopy. Anionic sites were randomly distributed along the length of microvilli. These sites did not show evidence of translational mobility within the membrane. In contrast, brush borders examined from animals at weaning (about 22 to 26 days) resembled those from the adult in which receptor sites were capable of mobility, and were induced by PCF to cluster into discrete patches. The random pattern of neonates was not affected by colchicine or cytochalasin B. It was altered by injections of cortisone which paralleled the premature cessation of pinocytosis and alternation of enzyme patterns described for glucocorticoids by others. It is suggested that the difference in mobility of anionic sites with age reflects the difference in absorptive function in the intestine of suckling and adult animals.

The Effects of Calcium Deficiency and Calcium Replacement by Strontium on Cytokinesis in Cultured Plant Cells. JESSE WOOD* and CHARLES W. GOFF, Indiana State University, Terre Haute, Indiana 47809._____The effects of calcium (Ca^{2+}) deficiency and calcium replacement by strontium (Sr^{2+}) on growth and cell plate formation in cell suspension cultures of *Dacus carota* were studied. Ca^{2+} deficiency has been shown to inhibit fusion and/or coalescence of the vesicles which form the cell plate. In the absence of Ca^{2+} , Sr^{2+} has been shown to "detoxify" a nutrient solution in a manner similar to Ca^{2+} , but Sr^{2+} is reported to have minimal nutritional value. *Dacus carota* were grown in a modified Murashiege-Skoog's media (Eriksson, 1965). The media water was distilled twice and run through a mixed bed deionizing column. The conductivity measured 0.9×10^{-6} MHO. Ca^{2+} deficiency was induced by omission of Ca^{2+} from the nutrient media. For media with Sr^{2+} replacing Ca^{2+} , Sr^{2+} was added at the concentration that Ca^{2+} normally is supplied (120 ppm). Short term effects for a four day period were monitored. Micrographs of Ca^{2+} deficient cells show

inhibition of cell plate formation as reported earlier. However, micrographs of cells which have Sr^{2+} replacing Ca^{2+} show no inhibition of cell division. Sr^{2+} treated cells continue to divide after repeated transfers into media with Sr^{2+} replacing Ca^{2+} . Whether Sr^{2+} substitutes for Ca^{2+} or simply controls the toxic effects (ion imbalance?) of Ca^{2+} deficiency, thus allowing normal function with extremely low levels of contaminating Ca^{2+} from the chemical stocks, is not known.

The Effects of Caffeine on Certain Growth and Metabolic Parameters in *Allium cepa* L. T. K. SPAULDING, S. P. BREHM and C. W. GOFF, Indiana State University, Terre Haute, Indiana 47809.——The effects of caffeine on the mitotic index (MI) and on macromolecular synthesis, and the rate of uptake, retention, and metabolism of caffeine by onion root tips were studied. Caffeine causes both long and short term reduction of the MI. This effect is concentration dependent and reversible. The optimal caffeine concentration (0.1%) caused a 40-50% reduction in the MI after 1 hr treatment and an 80% reduction by the end of 12 hr continuous treatment. No further reduction was noted at the end of 24 hr treatment. Roots treated for 3 hr with 0.1% caffeine, then placed in distilled water for recovery, showed a marked decrease in MI at the end of treatment but complete recovery within 3 hr. A marked inhibition in the rate of DNA and RNA synthesis was noted during a 3 hr treatment with 0.1% caffeine, but the rate of protein synthesis showed no significant difference when compared to control rates. The uptake, retention, and metabolism of caffeine were studied using H^3 -caffeine. Total uptake reached saturation levels within 1.25 hr. A 1 hr treatment with 0.1% caffeine followed by 3 hr continuous rinsing with distilled water showed no caffeine present at the end of 3 hr, but a marked presence of radioactivity. This suggests the caffeine was rinsed from the root and the radioactivity present in the post rinse homogenate represents incorporation of radioactive metabolites of caffeine. An attempt to identify these metabolites eliminated commonly accepted breakdown products of caffeine.

Novel Cells in the Uterus During Pregnancy. S. A. RHINE, Division of Medical Genetics, Methodist Hospital, Indianapolis, Indiana, A. MILUNSKY and P. STUBBLEFIELD, Harvard Medical School, Boston, Massachusetts and G. G. PALMER, Department of Medical Genetics, Indiana University, School of Medicine, Indianapolis, Indiana 46202.——A sampling device and laboratory procedures have been established for obtaining trophoblastic (fetal) tissue from the internal cervical os of the uterus during the first trimester of pregnancy. This potentiates the possibility of performing prenatal analysis concerning the genetic well being of the fetus via the endocervical canal as an earlier alternative to amniocentesis.

Phase contrast analysis of the tissue obtained consistently yields four types of cells which have not, to our knowledge, been described previously.

1. Epithelioid polygonal cells which usually occur in mosaic sheets.
2. Large singular amoeboid cells which actively phagocytize erythrocytes and cellular debris.
3. Small fibroblastic amoebocytes which migrate from the tissue fragments and occur in clusters with no obvious cell-to-cell contact.

4. Variable size, anucleate, multivacuolated giant 'cells', some which measure over 2100 μm in diameter.

Procedures have now been developed for the routine isolation of the cell types. Further study into the elucidation of their origin may yield new insights into the anatomy and physiology of early human pregnancy.

Lipophilic Monofunctional Aldehydes Inhibit Ferricyanide Reduction by Photosystem II of Spinach Chloroplasts

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Introduction

The Hill reaction, which measures photosystem II electron transport, was studied in 6% glutaraldehyde-fixed chloroplasts by Park (11), Hallier and Park (5) and Yoshida (17), in 0.5-1% glutaraldehyde-fixed chloroplasts by Oku *et al.* (10), and in 5% glutaraldehyde-fixed chloroplasts by Hardt and Kok (6, 7). In general it was found that even low concentrations of glutaraldehyde (<0.5%) inhibited photosystem I activity more severely than photosystem II activity, implying that photosystem I is more accessible in isolated chloroplasts.

The present study differs from previous studies in several aspects: (1) the effects of glutaraldehyde and other aldehydes was studied after a short incubation period (3 min.); and (2) both hydrophilic and lipophilic aldehydes were used. The purpose of this study was to find out if ferricyanide reduction by photosystem II could be inhibited selectively with aldehydes, as with thiols (15).

Materials and Methods

Chloroplasts were isolated from market spinach in 0.4M sucrose containing 0.05M NaCl (SN chloroplasts), as previously described (2). Likewise, chlorophyll was determined as in previous studies (2).

Electron transport in photosystem I and II was measured as O₂ evolution or uptake as previously described (2). Reaction mixtures for the various reactions are given in Table I. Tris-washed chloroplasts were prepared according to Yamashita and Butler (16).

All monofunctional aldehydes except formaldehyde were purchased from the Aldrich Chemical Company. Millimolar solutions were prepared fresh daily by adding the undiluted stock solutions to weighed bottles with a micropipette to obtain the desired weights of aldehydes to prepare solutions of desired concentrations. These solutions were protected from light by foil and kept on ice during use to minimize polymerization.

Glutaraldehyde, a bifunctional membrane cross-linking agent, was purchased from Sigma Chemical Company in individually sealed ampules.

Results and Discussion

It is well known (1, 2, 4, 5, 6, 7, 9, 10, 12, 17) that electron transport in spinach chloroplasts or algae is not affected by low concentrations (<0.5%) of glutaraldehyde or formaldehyde. Higher concentrations (>0.5-6%), however,

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TABLE I *The Effect of Monofunctional Aldehydes on Electron Transport in Spinach Chloroplasts.*

ALDEHYDE	Conc. (μ M)	Stimulation or inhibition of electron transport rates (%)				
		FeCN(+DBMIB) ^a	FeCN ^b	DMBQ(+DBMIB) ^c	PD _{ox} ^d	MV ^e
Paraformaldehyde	500	0	+ 4	+ 7	-	-14
Acetaldehyde	500	+ 3	-13	+ 7	-	- 4
Butyraldehyde	500	-20	+20	- 8	-10	-27
Benzaldehyde	500	-18	- 7	-21	+12	-50
Octylaldehyde	500	-30	+ 8	- 2	- 7	- 5

^aH₂O $\rightarrow$ FeCN(+DBMIB) control rates varied from 90-120 μ equiv./mg chl $\cdot$ hr; reaction mixture contained chloroplasts (50 μ g chlorophyll), 25 mM Tris-Mes, pH 8, 0.5 mM FeCN, and 2 μ M DBMIB.

^bH₂O $\rightarrow$ FeCN control rates varied from 100-200 μ equiv./mg chl $\cdot$ hr; reaction mixture contained chloroplasts as above, 25 mM Tris-Mes, pH 6, 250 μ M FeCN and 2 mM NH₄Cl.

^cH₂O $\rightarrow$ DMBQ(+DBMIB) control rates varied from 400-600 μ equiv./mg chl $\cdot$ hr; reaction mixture contained chloroplasts as above, 25 mM Tris-Mes, pH 7, 2 mM NH₄Cl; 3 mM MgCl₂, 0.75 mM DMBQ and 2 μ M DBMIB.

^dH₂O $\rightarrow$ PD_{ox}(+DBMIB) control rates varied from 450-650 μ equiv./mg chl $\cdot$ hr; reaction mixture contained chloroplasts and other reaction components as above except 0.5 mM PD and 0.5 mM FeCN in place of DMBQ.

^eH₂O $\rightarrow$ MV control rates varied from 500-700 μ equiv./mg chl $\cdot$ hr; reaction mixture contained chloroplasts as above, 25 mM Tris-mes, pH 7, 2 mM NH₄Cl, 3 mM MgCl₂, 0.5 mM Azide and 0.5 mM MV.

inhibit PS II and PS I activity. Hardt and Kok (7) have shown that preincubation with glutaraldehyde for at least 30 min. destroys plastocyanin. It is also assumed that the methylviologen site in PS I can be inhibited, since it is located on the outside of the thylakoid membrane. A number of studies (10, 17) demonstrated inhibition of the Hill reaction, but the inhibition site in PS II was not pinpointed with precision. In this study we chose to test a number of aldehydes with a progressively longer sidechain (1-12 carbons) on various PS II reactions to see if lipophilicity or bifunctionality produced more inhibition. The results varied, depending on the reaction tested, as shown in Table I, but aldehydes with 1-8 carbons gave no significant inhibition except benzaldehyde, which inhibited the H₂O $\rightarrow$ MV reaction up to 50%. Glutaraldehyde, a bifunctional reagent, which polymerizes rapidly to polymers of unknown molecular weight (8) can cross-link membrane components (13). As seen in (Fig. 1), the greatest effect was on PS II ferricyanide and p-phenylene diamine reduction in presence of DBMIB to block electron flow to PS I. It was not a very effective PS I inhibitor in the 3 min. incubation period employed in this study, since the H₂O $\rightarrow$ MV reaction was only inhibited 35-50%, at most. Dodecylaldehyde (Fig. 2), a lipophilic monofunctional reagent, gave better inhibition of methylviologen reduction in PS I and ferricyanide reduction in PS II, (70-85%) inhibition. Diphenylacetaldehyde, a lipophilic short chain monofunctional aldehyde (Fig. 3), stimulated methylviologen reduction in PS I, but selectively inhibited ferricyanide reduction by PS II in presence of DBMIB. A similar selective inhibition of ferricyanide reduction in PS II has been observed by us with lipophilic thiols, such as octane thiol (15).

Proof that the lipophilic aldehydes act selectively on electron transport, but not on water oxidation in PS II, is provided in Table II, using Tris-washed

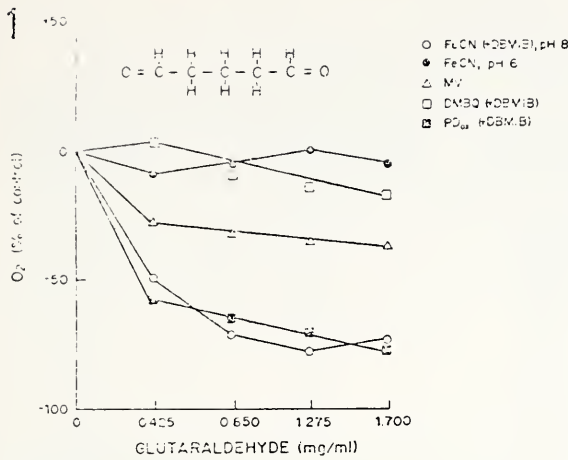


FIGURE 1. The Effect of Glutaraldehyde on Electron Transport in Spinach Chloroplasts. Reaction mixtures for the various reactions as in Table I. Control rates were as follows: 1020 $\mu\text{equiv.}/\text{mg chl} \cdot \text{hr}$ for $\text{H}_2\text{O} \rightarrow \text{MV}$; 176 for $\text{H}_2\text{O} \rightarrow \text{FeCN} (+\text{DBMIB})$; 182 for $\text{H}_2\text{O} \rightarrow \text{FeCN}$; 955 for $\text{H}_2\text{O} \rightarrow \text{PDox} (+\text{DBMIB})$ and 1072 for $\text{H}_2\text{O} \rightarrow \text{DMBQ} (+\text{DBMIB})$; + indicates stimulation of rate, — inhibition.

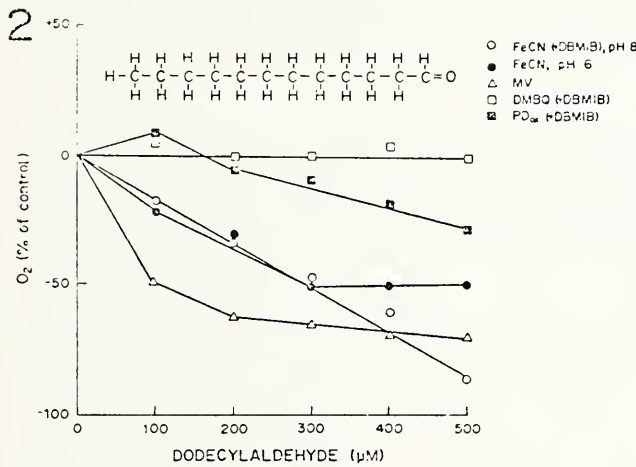


FIGURE 2. The Effect of Dodecylaldehyde on Electron Transport in Spinach Chloroplasts. Reaction mixtures and control rates as in (Fig. 1). + indicates stimulation, — inhibition of rate.

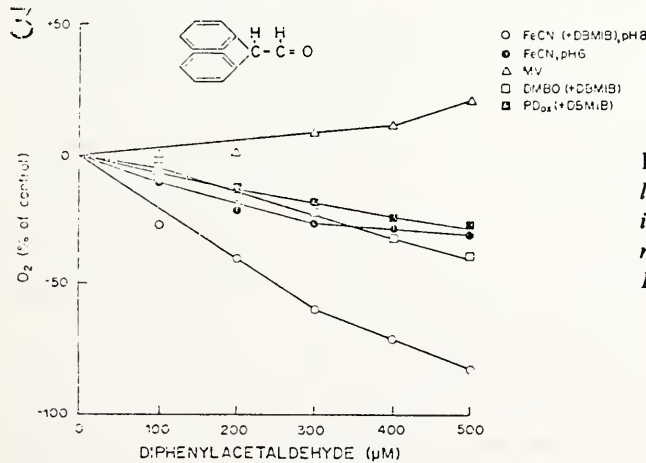


FIGURE 3. The Effect of Diphenylacetaldehyde on Electron Transport in Spinach Chloroplasts. Reaction mixtures and control rates as in (Fig. 1). + indicates stimulation, — inhibition of rate.

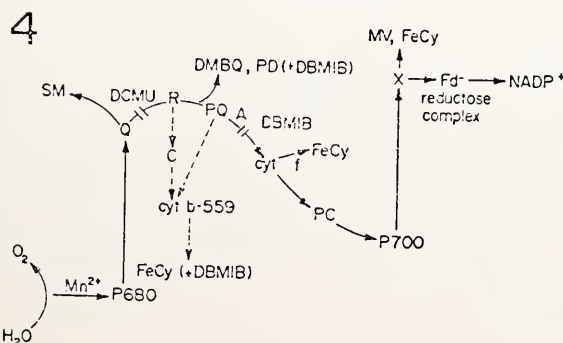


FIGURE 4. Electron Transport Scheme in Spinach Chloroplasts, Showing Possible Involvement of Cytochrome b-559 in Ferricyanide Reduction by Photosystem II.

TABLE II *Stimulation or Inhibition of the Diphenylcarbazine — DCPIP Pathway by Various Aldehydes in Tris-Treated Spinach Chloroplasts*

Aldehyde	Conc. (μ M)	Electron Transport Rate (μ equiv/mg chl · hr)	Stimulation or Inhibition (%)
Control Tris-washed Chloroplasts	-	72	0
Paraformaldehyde	500	76	+ 6
Acetaldehyde	500	76	+ 6
Butyraldehyde	500	75	+ 4
Benzaldehyde	500	60	- 17
Octylaldehyde	500	60	- 17
Glutaraldehyde	1.7 mg/ml	104	+44
Diphenylacetaldehyde	500	36	- 50
Dodecylaldehyde	500	24	- 66

¹Reaction mixture contained in 3 ml total volume: chloroplasts (50 μ g chlorophyll), 25 mM Tris-Mes, pH 7, 2 μ M NH_4Cl , 3 μ M MgCl_2 , 0.5 mM DPC and 0.5 mM DCPIP.

chloroplasts, in which water does not serve as electron donor any longer, but is substituted by diphenylcarbazine. As can be seen in this table, the effect of glutaraldehyde is different from the effect of the 2 more lipophilic aldehydes, diphenylacetaldehyde and dodecylaldehyde. Since glutaraldehyde stimulates the DPC $\rightarrow$ DCIP reaction, which involves only PS II, the inhibition observed with glutaraldehyde on the $\text{H}_2\text{O} \rightarrow \text{MV}$ pathway (Fig. 1) must involve sites in PS I, or an effect on water oxidation itself. Since PS I is located on the outside of the thylakoid membrane, cross-linking of outer membrane proteins by glutaraldehyde to give inhibition of electron transport in PS I is not unreasonable (13). Diphenylacetaldehyde and dodecylaldehyde, on the other hand, clearly inhibit electron transport in PS II. This indicates the monofunctional lipophilic aldehydes can penetrate the thylakoid membrane to reach sites located on the inner half of the thylakoid membrane, where PS II, including water oxidation, is located. Another possibility is that only that portion of PS II exposed to the outside, as shown by DABS labeling studies (3) is the segment affected. Since benzaldehyde and octylaldehyde only give a slight inhibition of PS II electron transport (17% on the DPC $\rightarrow$ DCIP pathway, Table II), compared to (>50%) inhibition by diphenylacetaldehyde and dodecylaldehyde, a more lipophilic compound than a straight-8-carbon chain skeleton is necessary to inhibit PS II electron transport.

The mode of action of the monofunctional lipophilic aldehydes in causing inhibition of PS II electron transport is unknown, but the possibility of interaction between iron-sulfur centers and these aldehydes exists, as was shown in mitochondria by Salerno and Ohnishi (14). Further studies are necessary to prove this point in chloroplast PS II.

Abbreviations Used

DBMIB — dibromothymoquinone; DCPIP — 2,6-dichlorophenol indophenol; DCMU — 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DMBQ — 2,5-dimethylbenzoquinone; DPC — diphenylcarbazine; FeCN^- — potassium ferricyanide; MV — methylviologen; PD — p-phenylene-diamine; PS I — photosystem I; PS II — photosystem II.

Summary

Various mono- and bifunctional aldehydes with chainlength from 1-12 carbon atoms affect electron transport in spinach chloroplasts. Glutaraldehyde, a bifunctional reagent, affected photosystem I activity, but lipophilic dodecylaldehyde inhibited both photosystems. Diphenylacetaldehyde, another lipophilic compound, inhibited specifically photosystem II activity, assayed by ferricyanide reduction in presence of dibromothymoquinone to block electron flow toward photosystem I. The localization of the two photosystems in the thylakoid membranes is discussed in view of their accessibility to aldehydes.

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Lipid Retention During Electron Microscope Preparation of *Penicillium chrysogenum*

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Introduction

For accurate visualization by electron microscopy of membranes and other structures with high lipid content, it is essential that a large percentage of lipid be retained during the fixation and dehydration process. It is also necessary to know the amount and distribution of labeled lipid lost in solvents during cell preparation.

It has been determined that lipids can best be preserved by using osmium-glutaraldehyde fixation process (3). Using this procedure Ainsworth reported a loss of only 0.7% to 7.2% total lipids in rat liver. In autoradiographic studies with hamster tissue, Dermer (1) found that a lipid loss of about 16% during preparation with osmium did not prevent localized radioactivity in specific intracellular structures. Stein and Stein (8) have shown that less than 20% ^{14}C -labeled total lipid was lost if the time for each alcohol and propylene oxide dehydration step was reduced.

These and other studies suggest that the degree of lipid retention depends upon the kind of intracellular lipid, the type of tissue and the technique in preparation (4-7).

Methods and Procedures

Young and aging submerged cultures of *Penicillium chrysogenum*, Q 176, were incubated with $1\text{-}^{14}\text{C}$ -palmitic acid according to procedures in our laboratory (6, 9). After 2, 10, 60, and 120 minutes incubation with labeled media, 20 ml samples of cultures were removed, filtered, washed with distilled water and subsequently processed for electron microscopy.

Mycelial cells were prepared for EM according to the procedure given in Table I. At each step of the fixation and dehydration schedule 0.1 ml of the washings was collected and the radioactivity determined by liquid scintillation spectrometry (Beckman ambient temperature liquid scintillation counter, Model LS-133). The amount of label incorporated into the mycelia in 20 ml samples of culture was determined by collecting other similar samples. The mycelia were filtered, washed, resuspended in 20 ml of 100% methanol and sonified for 2 min. The radioactivity of 0.5 ml of the sonicate was determined.

After the mycelia were prepared for electron microscopy, and just prior to embedding, total lipids were extracted from the treated mycelia and also extracted from similar samples of untreated mycelia (2). The lipids were separated into major lipid classes by thin layer chromatography using plates coated with silica gel G impregnated with 0.02% Rhodamine G solution using two solvent systems. (Solvent system I: n-hexane:ether:glacial acetic

TABLE I *Fixing, Dehydration and Embedding Procedure*

Reagent		
3% glutaraldehyde	2 hr	(1X)
cacodylate buffer, pH 7.2, 0.1 M	30 min	(3X)
cacodylate buffer, pH 7.2, 0.1 M	overnight	(or hold)
phosphate buffer, pH 7.3, 0.1 M	10 min	
phosphate buffer, pH 7.3, 0.1 M	overnight	
1% OsO ₄	2 hr	
ddH ₂ O	1 hr	
50% ethanol	2 min	(1X)
70% ethanol	2 min	(1X)
95% ethanol	5 min	(2X)
100% ethanol	5 min	(3X)
propylene oxide:resin; 1:1	overnight	infiltration
embedding resin	12 hr, 35 C	
	12 hr, 45 C	
	12 hr, 60 C	

acid: absolute methanol, 90:2:2:3, v:v:v:v) and Solvent system II; n-hexane: ether, 94:6, v:v). Bands were compared with standards (phospholipids, diglycerides, free fatty acids, triglycerides, cholesterol ester, and epoxy) scraped into scintillation vials and radioactivity was determined.

Results and Discussion

When the total amount of label extracted from the mycelia into the solvents was compared with that in untreated mycelia it was found that 6 to 7% of the total lipid was lost during electron microscope preparation (Table II). Although as much as 50% of these lipids were extracted in the alcohol changes, the propylene oxide dehydration extracted the largest amount of cell lipids of any single step. Aging cells tended to lose more lipids in alcohols. The large amount of lipids extracted from young cells by glutaraldehyde was not observed in aging cells (Fig. 1).

TABLE II *Extraction of Labeled Lipids by Solvents in EM Preparation*

	Young (pH 7.4)	Aging (pH 3.4)
Mycelia ^A	15,883 dpm ^B	41,200 dpm
Washes from mycelia	1,172 dpm	2,489 dpm
Percent loss	7.3%	6.0%

^AFrom 20 ml of culture.

^BDecompositions per minute.

Thin layer chromatographic analysis of lipid components of untreated mycelia revealed that the free fatty acid fraction represented the largest portion of lipid in aging cells. The amount recovered in young cells was negligible (Fig. 2). Aging cells not only accumulated lipids as pools of free fatty acids but distribution of label indicated that aging cells incorporated large amounts of the

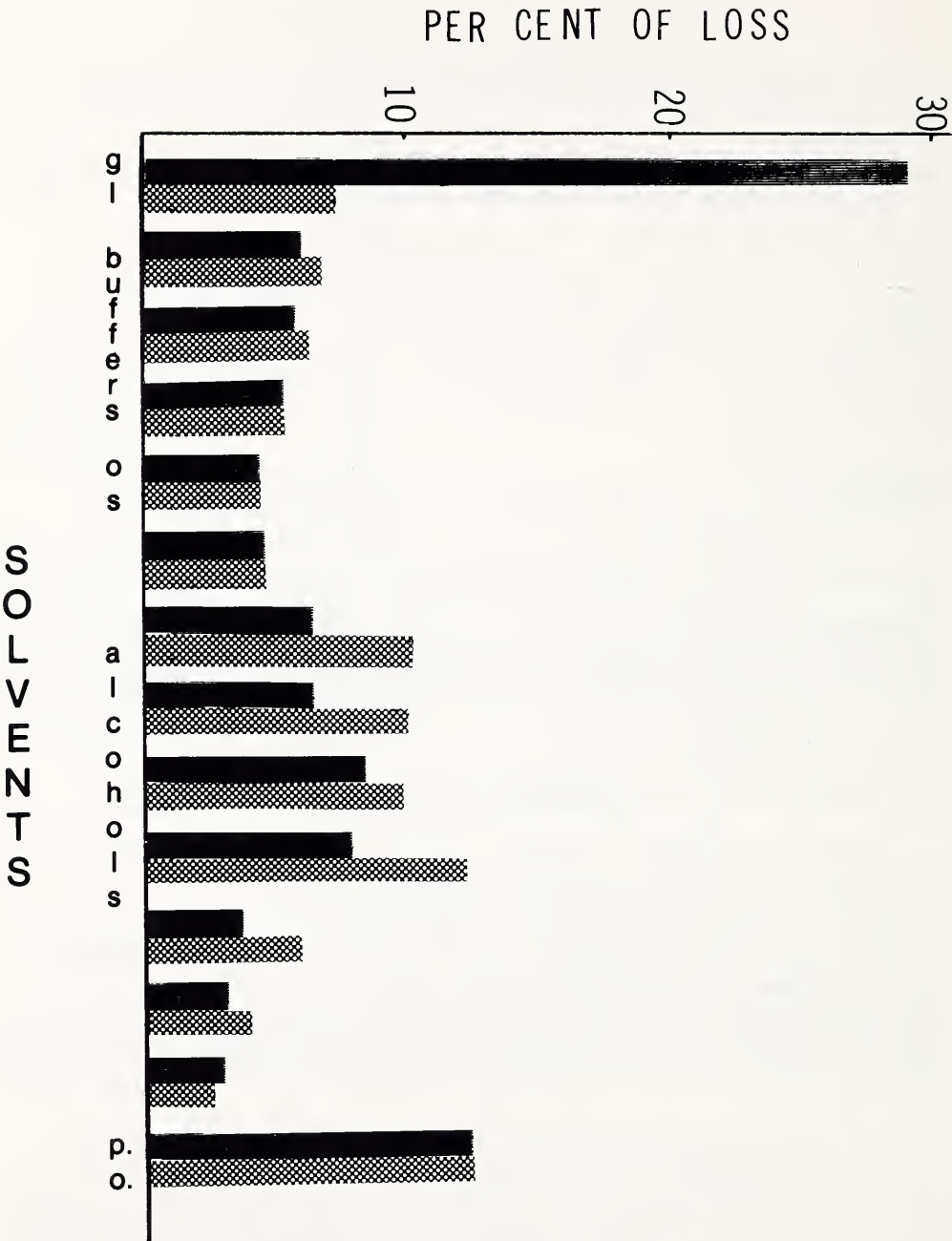


FIGURE 1. Extraction of Labeled Lipids by Solvents in Electron Microscopy Preparation. gl—gluteraldehyde, os—osmium tetroxide, p.o.—propylene oxide. Young, Aging.

labeled palmitic acid into free fatty acids also. In untreated cells, a maximum of 74% of the label was recovered in the free fatty acid fraction after 2 minutes. At all other incubation times approximately 70% of the label was recovered from the free fatty acid fraction. Smaller amounts of other lipid components were present; phospholipids comprised about 20% at all time points. In aging cells treated for electron microscopy, more than half of the incorporated label was extracted in the dehydration procedure. Large amounts of the free fatty acids



FIGURE 2. Distribution of Lipids from Young and Aging Cultures of *Penicillium chrysogenum*. PL—phospholipids, DG—diglycerides, FFA—free fatty acids, TG—triglycerides, CE—cholesterol esters.

were lost; the percentage dropped from 70% to only 15% after EM preparation. Phospholipids were retained in aging cells; all other lipid components remained approximately the same (Table III).

TABLE III Effect of EM PREP on Distribution of Label in Lipid Classes (Aging Cultures)

Time ^A	Percent Label Incorporated Into:					
	PL	DG	FFA	TG	CE	EP
2 min before ^B after	15	6	74	4	1	
	33	14	13	13	13	14
10 min before after	20	8	64	6	2	
	45	12	11	11	10	11
60 min before after	19	5	68	5	3	
	43	11	12	10	11	13
120 min before after	21	4	72	3	-	
	40	11	12	12	13	12

^ATime after addition of label
^BBefore and after EM PREP

Young cells, unlike aging cells, incorporated label primarily into the phospholipids with smaller amounts in other lipid components. Young cultures prepared for electron microscopy retained most of the labeled phospholipids throughout the solvent treatments. The percent of phospholipids in the distribution dropped from 70% to about 50% in treated cells. In young cells about 15% of the label was found in the epoxy resin, indicating little label loss in the other lipid components (Table IV).

TABLE IV *Effect of EM PREP on Distribution of Label in Lipid Classes (Young Cultures)*

Time ^A	Percent Label Incorporated Into:					
	PL	DG	FFA	TG	CE	EP
2 min						
before	59	10	13	12	6	
after	42	10	14	11	11	12
10 min						
before	55	9	15	15	6	
after	34	11	14	12	13	16
60 min						
before	73	4	11	11	1	
after	34	11	15	13	14	13
120 min						
before	80	3	15	2	-	
after	67	6	8	6	6	7

^ATime after addition of label

Conclusions

Retention of total lipids appeared adequate for electron microscope autoradiography in both young and aging cells of *Penicillium chrysogenum*. However, unbound lipids, such as free fatty acids, were highly soluble in the solvents and tended to be lost from the cells during electron microscope preparation. Distribution of labeled lipids was altered considerably in the aging cells because of their high free fatty acid content. These changes in distribution should be taken into consideration in autoradiographic analysis of cells characterized by large lipid reserves.

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Antigenicity of Solubilized Protein From Cells Infected with Pseudorabies Virus

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Introduction

Solubilization of biological membrane proteins for the investigation of their functional properties and structure is at the present time best achieved by using non-ionic detergents. Triton X-100 (polyoxyethylene p-t-octyl phenol), a non-ionic detergent, has been recognized as one of the most effective solubilizers of biological membrane (1, 5, 6, 7, 15, 17). Analysis of the solubilized proteins in the presence of the non-ionic detergent may cause difficulties, such as loss of biological activity. However, immunoelectrophoretic analysis in the presence of this detergent is possible because the antigenicity of the membrane protein is retained after solubilization with non-ionic detergent (2, 15). Although little exact data is presently available on the mechanisms by which this detergent acts, it is possible that due to selective binding of the non-ionic detergent to the hydrophobic part of the membrane protein, the antigenic determinants are left unaffected and free to react with antibodies.

Quantitative immunoelectrophoresis has been used for analysis of soluble proteins obtained from erythrocyte membranes (4). Crossed immunoelectrophoretic analysis of soluble proteins of herpes simplex viral infected cornea cells (rabbit) has also been described (3). This is to report on the identification and quantitation of a water soluble, membrane bound glyco-protein viral antigen.

Materials and Methods

Pig kidney cells (PKW2E) derived from 3-week-old pigs, at the 90th passage level, were used in the studies. The cells were grown in flasks with Eagle's medium containing 10% fetal calf serum (FCS), 100 U of penicillin per ml and 170 μ g of streptomycin per ml at pH 7.4 and maintained in a similar medium containing 2% FCS. The strain of pseudorabies virus, PrV-FH, was used throughout the study. Plaque assay was done in PKW2E cells by determination of P.F.U. (Plaque forming units/ml) at 48 hours post exposure.

Triton X-100 (J. T. Baker Chemical Company, Phillipsburg, New Jersey 08865) was the detergent used. The cell debris after viral harvest was suspended in 0.01 M glycine — 0.0038 M Tris buffer pH 9.0, with and without detergent at a volume of six times that of the packed cellular debris. The mixture was sonically treated in an ice bath at 10 kc/sec three times for 15 seconds each (Branson Sonifer B12 with microtip, Danbury, Connecticut). The pellet obtained after centrifugation at 4°C in 60-T₁ rotor for 2 hours at 50,000 r.p.m. was dispersed in the same buffer and treated as above. This was repeated twice. The final pellet of cellular debris obtained after the third treatment was solubilized under special conditions (with or without detergent as shown in Table I).

TABLE 1 *Percent of Solubilized Protein from PrV-Infected Cells with Different Solubilization Procedures*

Solubilization procedure	Buffer without detergent		Buffer = 2% Triton X-100		Buffer without detergent (for extraction) Buffer + 2% Triton X-100 (for final pellet)	
	Solubilized protein (mg/ml)	Percent of solubilization (%)	Solubilized protein (mg/ml)	Percent of solubilization (%)	Solubilized protein (mg/ml)	Percent of solubilization (%)
First*: Sonication of 1 g. (wet wt.) cell pellet in 6 ml. fluid	4.0	41.1	5.5	50.0	5.0	39.2
Second*: Sonication of pellet of first in 2 ml. fluid	4.5	15.4	5.0	15.2	5.25	13.7
Third*: Sonication of pellet of second in 2 ml. fluid	4.25	14.6	4.5	13.6	5.0	13.1
Total extent of solubilization (%) in supernatant	—	~71	—	~79	—	~66
Fourth**: Dissolving the final pellet in 2 ml. fluid	0.42	28.9	0.35	21.2	0.65	34

*Fractions: First, Second, and Third are the supernatant fluid after centrifugation at 50,000 r.p.m. for 2 hr.

**Fraction: Fourth is the solution of final pellet dissolved in the fluid without centrifugation

Standard viral protein or other protein concentration was determined by biuret method (10) with bovine serum albumin as a standard.

After sonic treatment of viral infected cellular debris, the solubilized protein was purified by a modified method described by Ritzi et al (13). The soluble protein was applied to a washed column of concanavalin A-sepharose. The solubilized protein was eluted from the column with successive washes of 50 and 100 mg of methyl-D-mannoside (Sigma Chemical Company) per ml and dialyzed against 0.02 M Tris (pH 7.8). The dialyzed sample was then concentrated with Aquacide II (Calbiochem) and applied to a DEAE cellulose column. The protein was eluted from the column with increasing concentrations of NaCl (0-0.3 M). The protein fraction was finally dialyzed against distilled water and concentrated with Aquacide II. This purified protein was kept at -90°C and identified by immunodiffusion and rocket immunoelectrophoretic analysis.

A specimen of serum from immune swine was used as a source for antibody purification. A crude globulin fraction was separated from the serum by precipitation with concentrated ammonium sulfate (final molarity 1.77 M). The resultant slurry was centrifuged and passed through a column of coarse Sephadex G-25 as described by Kanitz (9). The eluent from the column was finally concentrated 10-fold with Aquacide II.

Immunodiffusion and rocket immunoelectrophoretic analyses were performed by using the procedure described by Sun et al (14).

Results

The amount of protein solubilized from the PrV-infected pig kidney cells by using different procedures is illustrated in Table I. The triton X-100 detergent treatment resulted in a higher total extent of solubilization in supernatant (~79%) as observed in percent of solubilization of column II (combined first, second and third fractions) in comparison to those of column I and column III (~71% and 66% respectively) which were extracted three times in buffer alone (without detergent). Treatment of the final pellet (fourth fraction) with 2% Triton X-100 also resulted in an additional (~5%) solubilization from cellular debris (34% in comparison to 28.9%). These results indicate Triton X-100 decreases protein-membrane binding affinity and releases protein.

Table II shows the difference in biological activity between proteins solubilized with and without the use of Triton X-100. A small amount of protein would also be solubilized in the presence of buffer alone as observed in Table I. Immunodiffusion analysis of the crude extract was used to compare the antigenicity of soluble fractions from the two solubilization procedures (with or without detergent). Extraction of antigenic determinants seemed to be a process requiring Triton X-100. There was no immunoprecipitate formed in the agar gel when buffer alone was used in the solubilization procedure (Table II). On the contrary, well-defined immunoprecipitate was formed in several dilutions in the presence of anti-PrV swine serum when the detergent was used in the procedure. This study also indicates that the antigenic protein is tightly bound to cell membranes.

TABLE II *Comparable Analyses of Antigenicity of Fractions from Different Solubilization Procedures by Immunodiffusion*

Solubilization procedure		Immunodiffusion				
		Undil.	Dilution			
			1:2	1:4	1:8	1:16
Buffer without detergent	Supernatant	—	—	—	—	—
	Final pellet	—	—	—	—	—
Buffer + 2% Triton X-100	Supernatant	++	+	+	—	—
	Final pellet	—	—	—	—	—
Buffer without detergent (for extraction) Buffer + 2% Triton X-100 (for final pellet)	Supernatant	—	—	—	—	—
	Final pellet	+++	++	+	—	—

Immunodiffusion test was performed by filling the center well with anti-PrV serum and the surrounding wells with dilutions of materials obtained from different solubilization procedures.

- +++ indicates a very strong positive precipitin band;
- ++ indicates a strong positive precipitin band;
- + indicates a weak positive precipitin band and
- indicates a negative reaction.

To analyze the soluble protein fraction further, purification of the crude extract through columns of concanavalin A-sepharose and DEAE cellulose was performed consecutively. Approximately 50% of the absorbing material of optical density at 280 nm (O.D. 280) from the solubilized crude extract was retained on DEAE cellulose column. By using a narrow NaCl gradient from 0 to 0.3 M, a single peak of 0.d. 280 absorbing material (fractions 3 through 7 in Fig. 1) was obtained in the NaCl gradient 0.047 M region (Fig. 1) with Gilford Instrument, Model 2400-S. This fraction was ready for assay of the antigenicity by immunodiffusion and rocket immunoelectrophoresis.

Analysis of the above single protein peak for the antigenic reactivity by immunodiffusion is shown in (Fig. 2). Anti-PrV swine serum in well 7 reacted with material in wells 2, 4, and 6 (filled with purified fraction of detergent solubilized protein) as it did with material in wells 1, 3, and 5 (filled with control positive antigen). This arrangement provided a control precipitin line on each side of the tested sample. The control line joined with the line of precipitation between the tested sample and specific serum forming a hexagonal ring. The antigenic protein in the Triton X-100 solubilized fraction was therefore identified as an antigen being homologous with or similar to the control positive antigen.

When normal pig serum was placed in the center well, lines of precipitation were not obtained, ruling out the possibility of non-specificity in tests of the antigen with antibody containing sera. The result realized when the solubilized

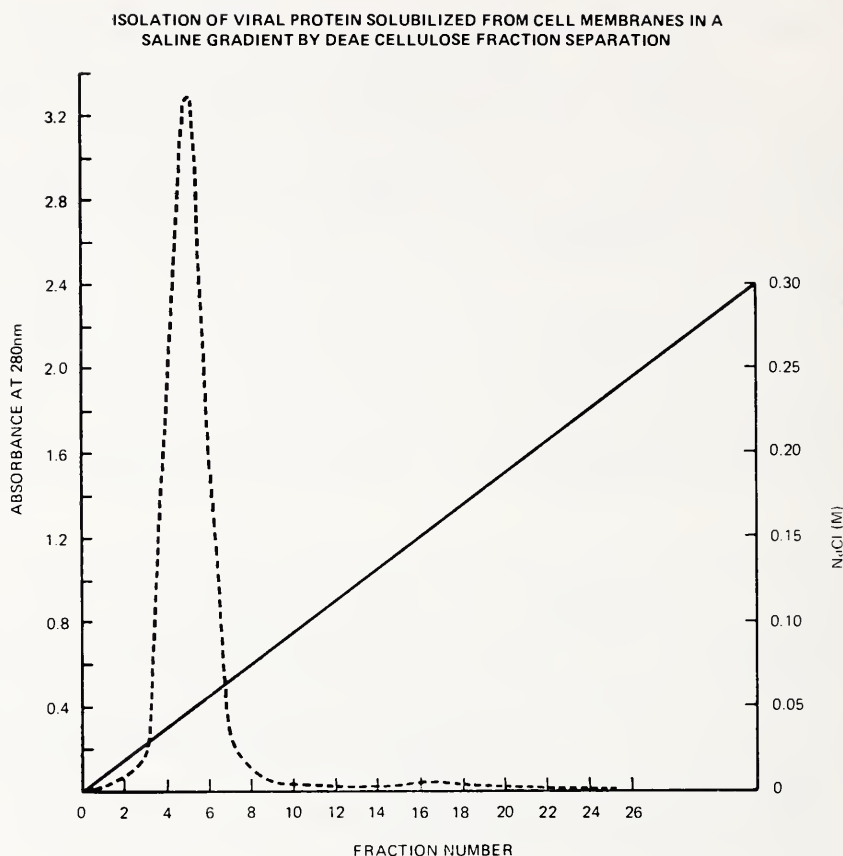


FIGURE 1. Isolation of viral protein solubilized from cell membranes in a saline gradient by DEAE cellulose fraction separation. The solubilized crude extract was first applied to concanavalin A-sepharose column (1.5 by 25 cm) equilibrated in the buffer (methyl-D-mannoside, 100 mg/ml). 150 ml of eluent was collected after sample application. The eluent was dialyzed, concentrated, and applied to DEAE cellulose column with a 600 ml gradient of 0 to 0.3 M NaCl. The absorbancy at 280 $m\mu$ was determined immediately.

protein obtained without the use of the detergent, as shown in (Fig. 3), was non specific since the precipitation lines between wells 2, 4, and 6 did not form a continuous line with those of the control wells 1, 3, and 5.

Antigenic determinants from above preparations (with or without Triton X-100) were investigated and quantitated with rocket immunoelectrophoretic techniques. Plot of immunoprecipitate migration distances of PrV dilutions from rocket immunoelectrophoresis was shown in (Fig. 4). This was used to provide a standard curve. The length of the precipitate is directly proportional to the concentration of the antigen. To obtain a quantitative estimate of the antigenic protein in the unknown sample, the length of the immunoprecipitate (E. G. the upper edge of the application well to the peak) was measured. By comparing the value obtained to that of the standard curve, the concentration of the antigenic determinant within the test sample was determined. (Fig. 5) shows that purified and solubilized protein extracted with Triton X-100 give a well-defined, rocket-shaped immunoprecipitate (well B). A similar phenomenon was not observed in well C which was filled with purified and solubilized protein

extracted without Triton X-100. The length of the precipitate shown in well B (50 λ of test sample) compared with that of well A (54 λ of standard control antigen) and the standard curve in (Fig. 4), shows the concentration of antigenic protein in well B to be 9.6 mg/ml.

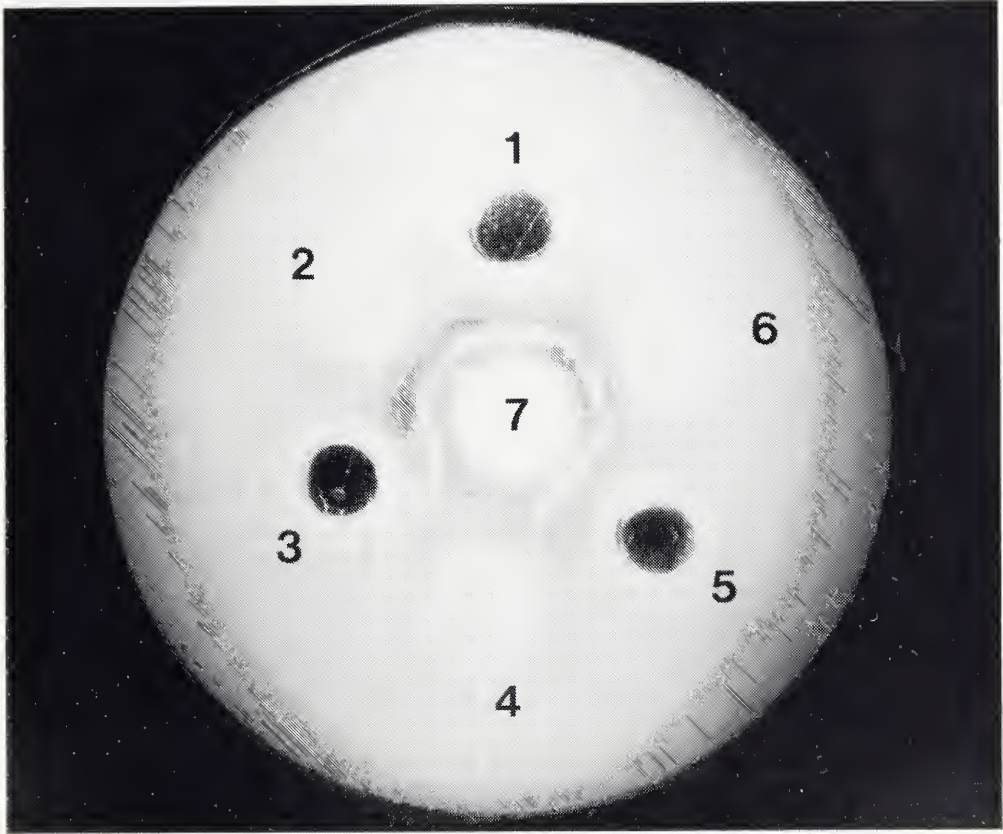


FIGURE 2. Identification of the purified protein fraction of detergent solubilized membrane protein by immunodiffusion. An equal volume (40 microliters) of control positive antigen and test sample was present in wells. Wells represented in 1-7 are the following: (1), (3) and (5) control positive antigen; (2), (4) and (6) purified protein fraction of Triton X-100 solubilized membrane protein.

Discussion

Presently, very little exact data are available on the mechanism by which non-ionic detergents act. Our studies using Triton X-100 as a membrane surfactant to solubilize tightly membrane-bound viral proteins and retain their antigenicity suggests that the detergent does not cause complete disruption of the membrane. The most likely mechanism involved is that the viral protein-cellular membrane binding affinity change is due to a conformational change in the structural proteins of the membrane.

In order to get optimum precipitate length reproducibility in rocket immunoelectrophoresis, the following factors are important: (a) antibody concentration, (b) field strength (volt), (c) buffer pH or ionic strength, (d) gel concentration, (e) time of electrophoresis, and (f) time between sample application and the initiation of electrophoresis. Variation in any of the above factors makes reproduction of the experimental data impossible.

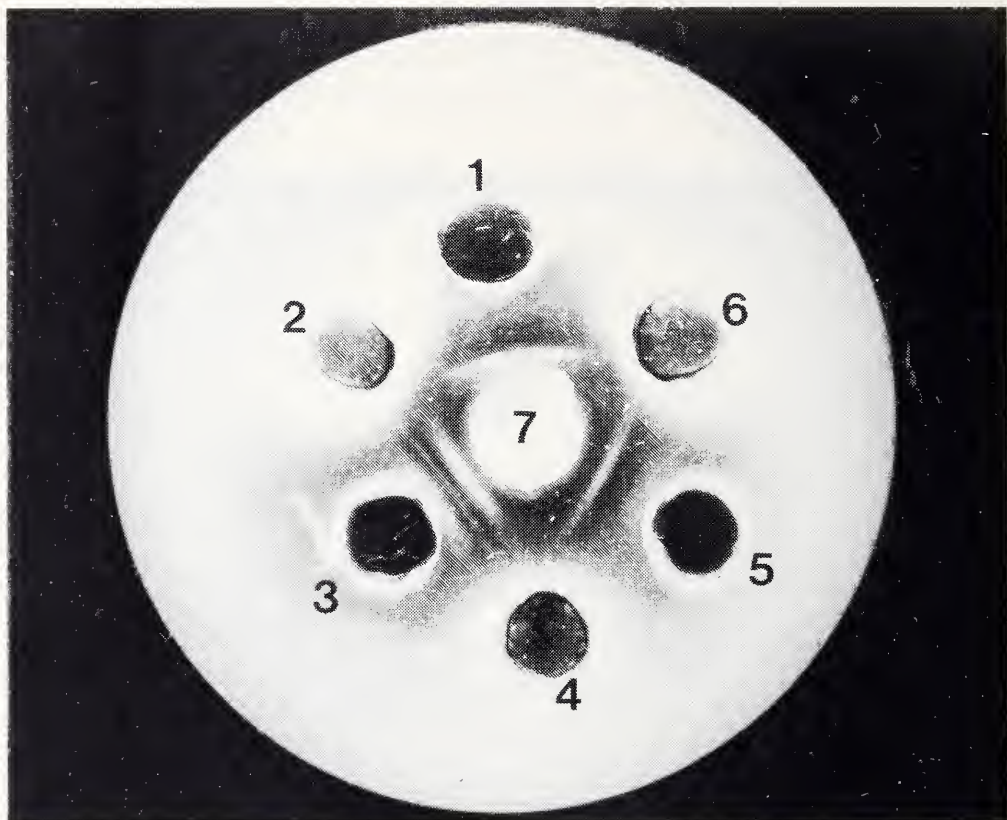


FIGURE 3. Immunodiffusion analysis of purified protein fraction obtained with buffer alone (without detergent). An equal volume (40 microliters) of control positive antigen and test sample was present in the wells. Wells represented in 1-7 are the following: (1), (3) and (5) control positive antigen; (2), (4) and (6) purified protein fraction of buffer (without detergent) solubilized protein; (7) anti-PrV swine serum.

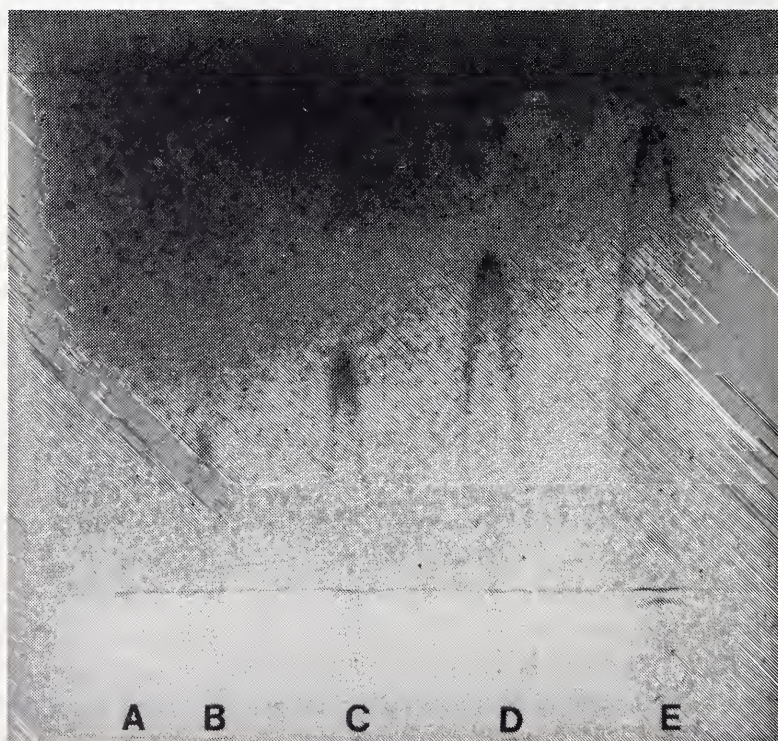


FIGURE 4.

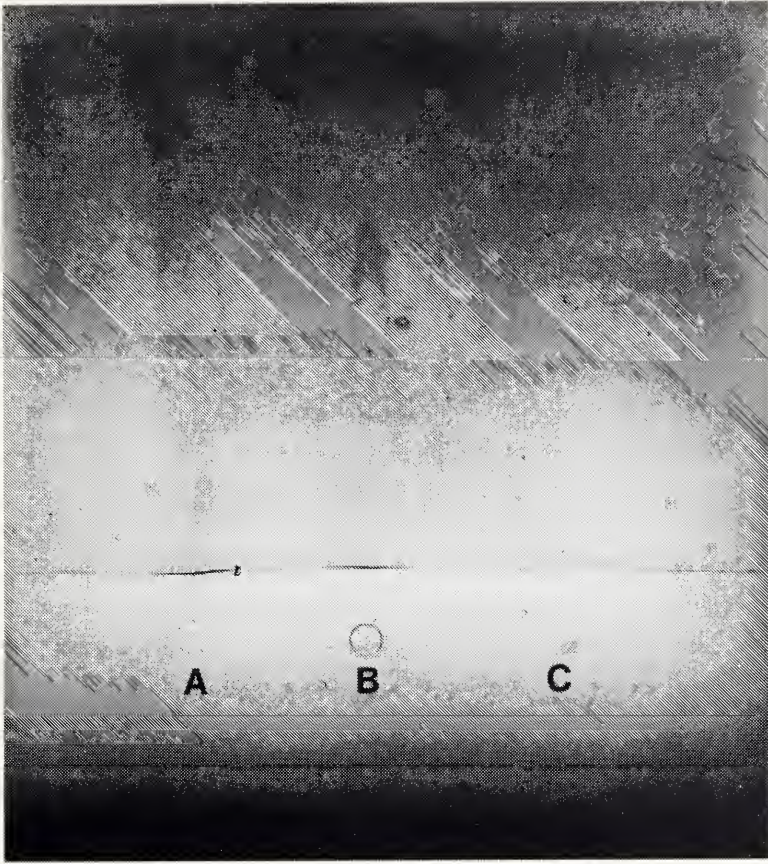


FIGURE 5.

Since the specificity of binding the detergent solubilized protein to anti-PrV swine serum appears to be the same as that observed with positive control antigen (Fig. 2 and Fig. 5), it is postulated that Triton X-100 required soluble protein represents either all or some portion of receptor for anti-PrV gamma-globulin. A similar phenomenon was observed by others previously (15, 11) in herpes simplex virus infected rabbit cornea cells and mouse tumor cells respectively. The fact that the O.D. (optical density) reading in 280 nm and 260 nm for the detergent solubilized protein has a ratio different from that of the purified standard virion ($\text{O.D. } 280 \text{ nm} / \text{O.D. } 260 \text{ nm} = \text{protein/nucleic acid} = 1.33$) indicates that this soluble protein is not the intact virion but a part or subunit of the virion.

Our investigation as well as Hoggs (8) disclose the existence of an antigenic protein on the cell surface which is derived from the replicative process for infectious virus used in the infection of cell culture. It is probably the result of adsorption of a viral component to the cell surface, although more complex interactions which result in changing the plasma membrane properties are possible. A similar mechanism was proposed previously by Phillips and Perdue (12) in which the ability of binding foreign protein to cell surface resulted in a plasma membrane characteristic which permitted transformed and untransformed cells to be distinguished from one another.

The application of detergent solubilized herpes simplex viral antigen to produce neutralizing antibody in rabbits has been reported (15). It may be

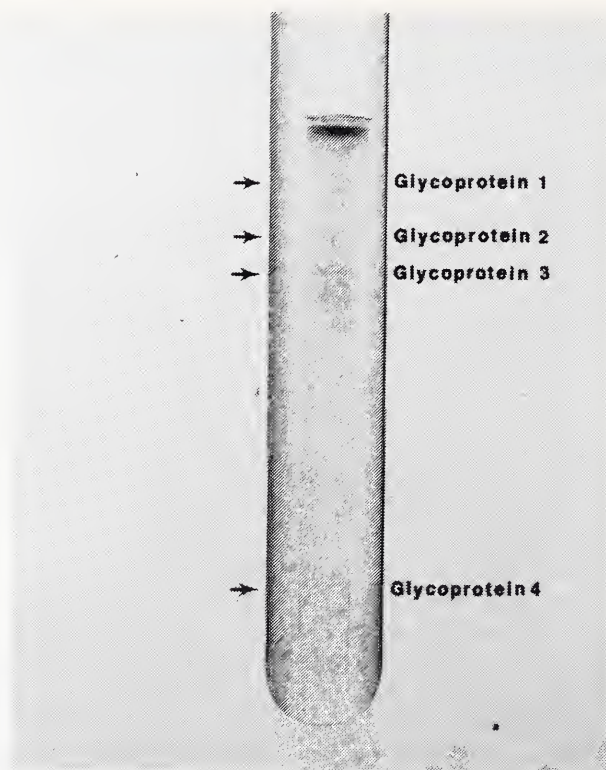


FIGURE 6. SDS polyacrylamide gel electrophoresis of DEAE cellulose column fraction. The gel was stained with Coomassie brilliant blue and the protein sample present in the gel was 100 μ l of protein solution (1 mg/ml).

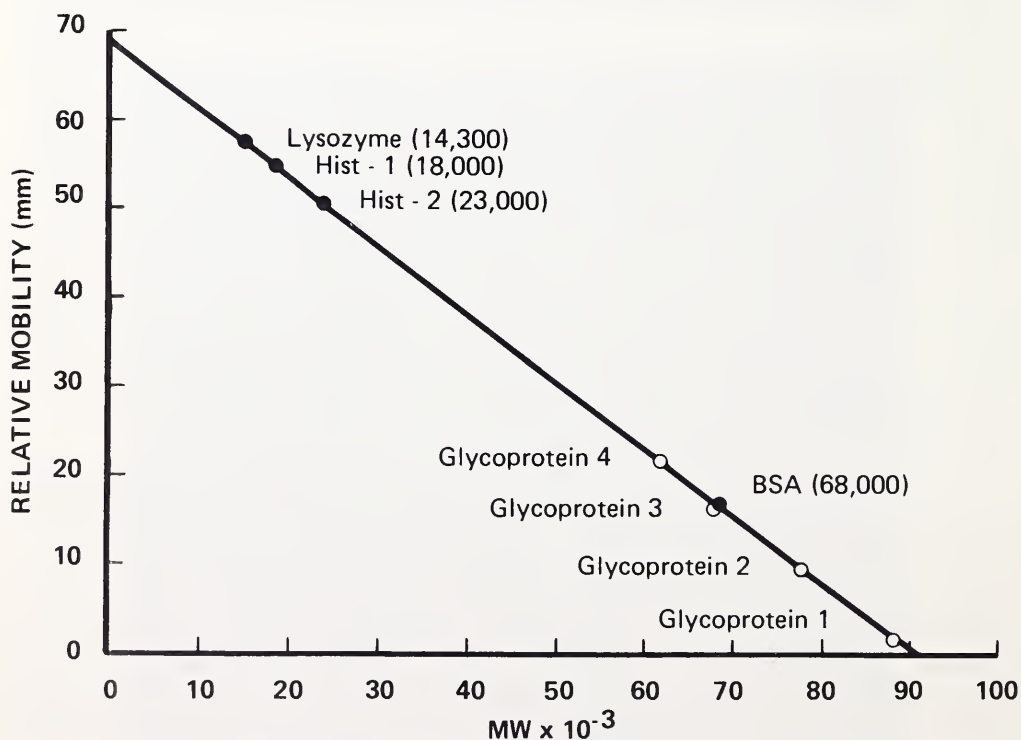


FIGURE 7. Relation between relative mobility and molecular weight for standard protein markers and the solubilized protein in SDS gel. Standard protein markers used for molecular weight determination included bovine serum albumin (68,000), histone-1 (23,000), histone-2 (18,000) and lysozyme (14,300).

possible to use the solubilized and Triton X-100 requiring PrV antigen as a subunit vaccine in animals.

There has been little experimental evidence to prove or detect persistent PrV infections in swine. Detection of persistent infections in swine may be possible by identifying the detergent solubilized protein extracted from cells of CNS ganglia through rocket electrophoresis.

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Histological Observations of Hepatocellular Carcinomas of Rats Fed Varying Amounts of Vitamin A¹

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Introduction

Vitamin A is known to be required in the normal pathway of epithelial cell differentiation in essentially all of the epithelial target sites of origin of cancer (4, 9). Carcinogenesis involves a de-differentiation or at least an aberrant differentiation in these epithelia. Vitamin A used in pharmacological amounts in cancer prevention is an attempt to arrest or reverse a pathological process by enhancement of the physiological processes of differentiation (8).

Many investigators have postulated that the phosphate ester of retinol, retinyl phosphate, may function in the glycosylation of glycoproteins (See 6 and 10 for literature). While it is premature to accept a metabolic function of vitamin A in connection with the transfer of mannose, galactose or other sugars to glycoprotein, it would be of interest if such a reaction were one function of the vitamin. This connection might be facilitated by a better understanding of the cellular basis of vitamin A effects. In this study, we evaluated histochemically the effects of vitamin A on glycoprotein synthesis in liver and tumor tissue with the aid of periodic acid-Schiff (PAS) reagent.

Materials and Methods

Weanling male rats of the Charles River strain were randomly divided into 3 groups of 10 each and fed diets containing no vitamin A, adequate amounts or excessive amounts of vitamin A (100X adequate) (Table I). Vitamin A was in the form of retinyl acetate. Food intake and weight gain were recorded weekly. Rats were fed respective diets for two weeks prior to being subcutaneously injected with transplantable hepatocellular carcinomas of the rat derived from solid tumors induced by N-2-fluorenylacetamide. Animals were examined daily for tumors. Tumor mass was estimated from weekly measurements with a vernier caliper of two tumor dimensions. The following mathematical formula was used to determine approximate tumor mass: $M = 4/3 \pi (a^2/4)b/2$ where a and b are the two dimensions (perpendicular axes) measured and M = mass (7).

Vitamin A concentration of liver and tumor mass was determined using the Bayfield (1) procedure. Protein concentration was determined for liver and

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TABLE I *Composition of basal diet used in tumor growth studies*

Ingredient	Percent
Casein	20.0
DL-Methionine	0.3
Cornstarch	15.0
Sucrose	50.0
Fiber (Cellulose Type)	5.0
Corn Oil	5.0
Mineral Mix	3.5
Vitamin Mix	1.0
Choline Bitartrate	0.2

tumor mass by the Bradford (2) Coumassie Blue dye binding assay. For histological analysis, portions of the tumor tissue were fixed in 10% neutral buffered formalin, embedded in Paraplast and reacted with PAS reagent and counterstained with hematoxylin. Mitotic index was determined by using an ocular grid in a microscope and counting all of the cells which fell within the grid spaces. Then cells undergoing mitosis were counted in the same field. Mitotic index equals number of mitotic cells observed divided by the total number of cells.

Results

Tumors were fewer and appeared later under conditions of either vitamin A deficiency or excess. By day 21 post-injection, 10% of the animals in the group fed adequate amounts of vitamin A had tumors and by day 28, 90% of the animals in this group exhibited tumor growth, whereas in both the deficient and excess vitamin A groups there were no signs of tumors at day 21 with only 10% of the animals in both groups having tumors at day 28. Diets containing excess amounts of vitamin A thereafter continued to prevent tumor growth to a greater extent than did the diets containing no vitamin A. On day 42, 50% of the animals fed the vitamin A-free diets and 40% of the animals fed the excess vitamin A diets exhibited signs of tumor growth. However, two of the animals fed excess vitamin A diets had tumors that were too small to measure with the vernier calipers. The rats that did not have tumors by day 42 were allowed to continue on their respective dietary regimen for approximately another 100 days and did now show signs of tumor growth during the duration of the experiment. Liver vitamin A approximately paralleled intake while that in tumors reflected but did not parallel the amounts fed.

Mitotic indexes (Table II) were highest for the adequate group, lower for the vitamin A deficient group and lowest for the excess vitamin A group. Tumor

TABLE II *Mitotic indexes of hepatocellular carcinomas of rats fed varying amounts of vitamin A*

Dietary Vitamin A Treatment Group	Mitotic Index
Deficient	0.055 $\pm$ 0.003*
Adequate	0.085 $\pm$ 0.018
Excess	0.045 $\pm$ 0.010

*Mean $\pm$ SD

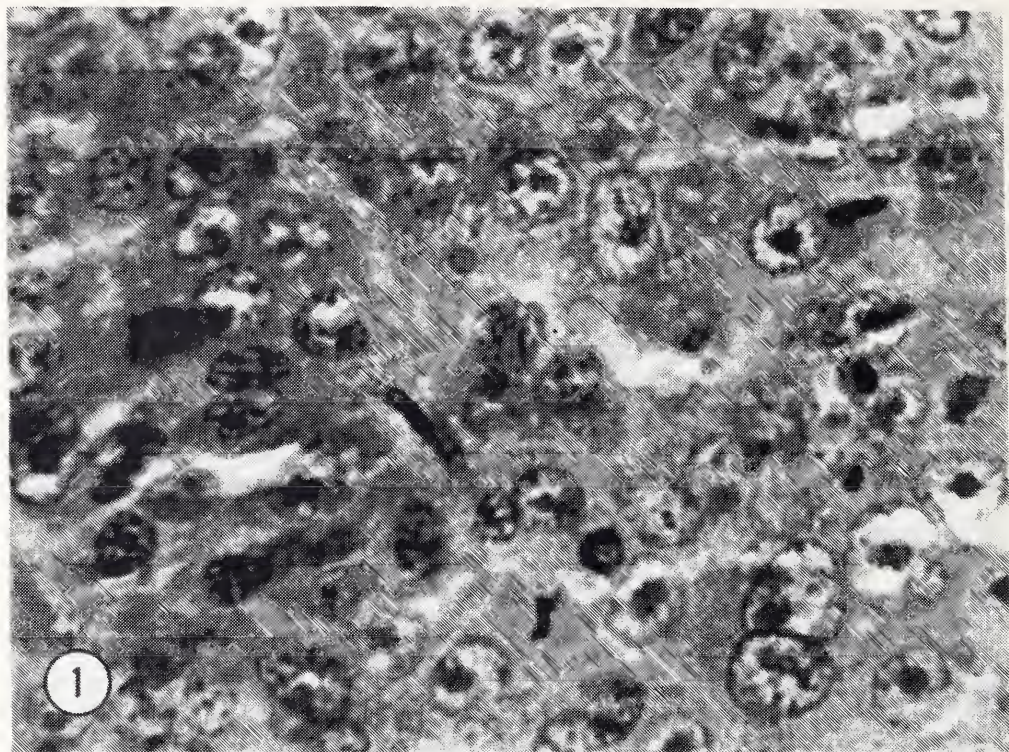


FIGURE 1. *Hepatocellular carcinoma of a rat fed an excessive amount of vitamin A. Mag. = 800X.*

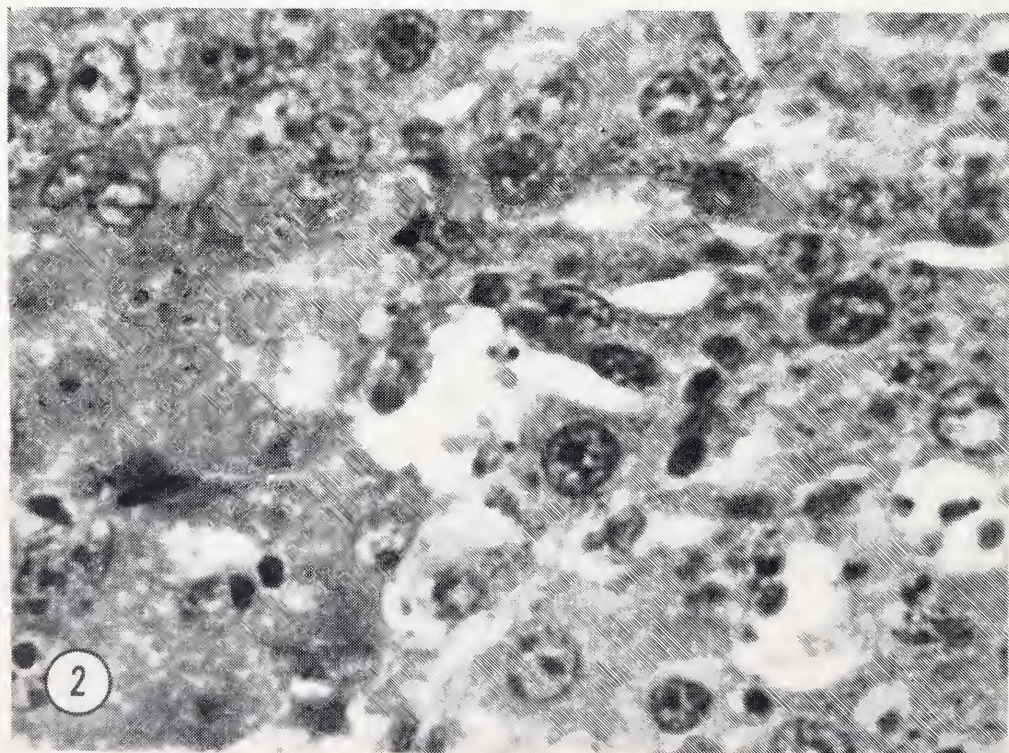


FIGURE 2. *Hepatocellular carcinoma of a rat fed an adequate amount of vitamin A. Mag. = 800X.*

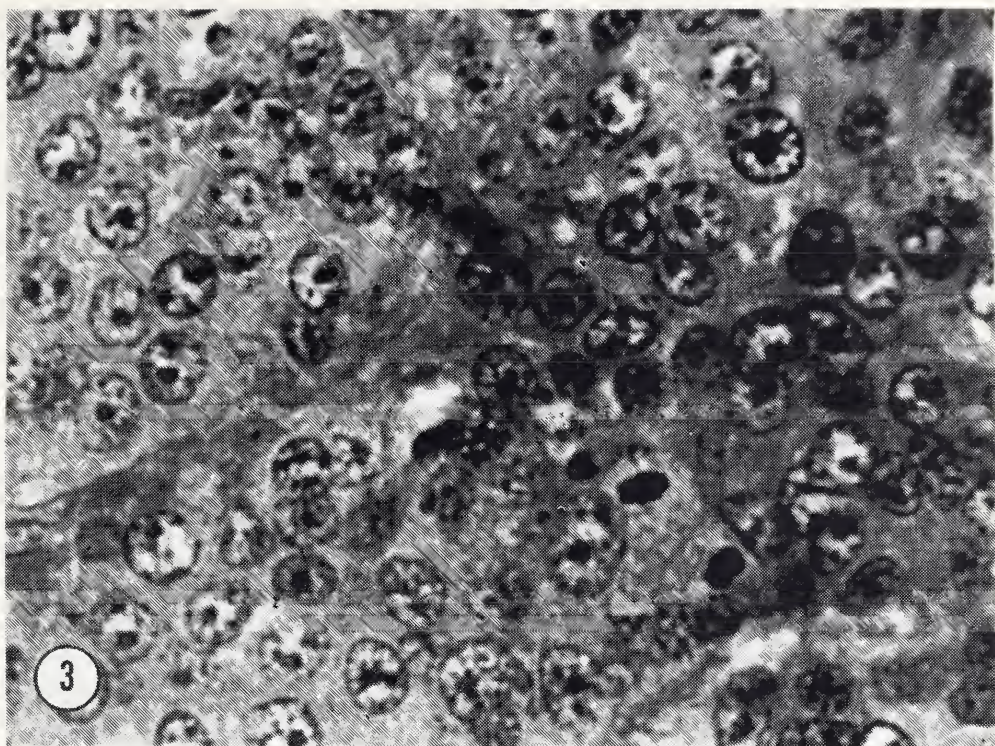


FIGURE 3. *Hepatocellular carcinoma of a rat fed a deficient amount of vitamin A. Mag. = 800X.*

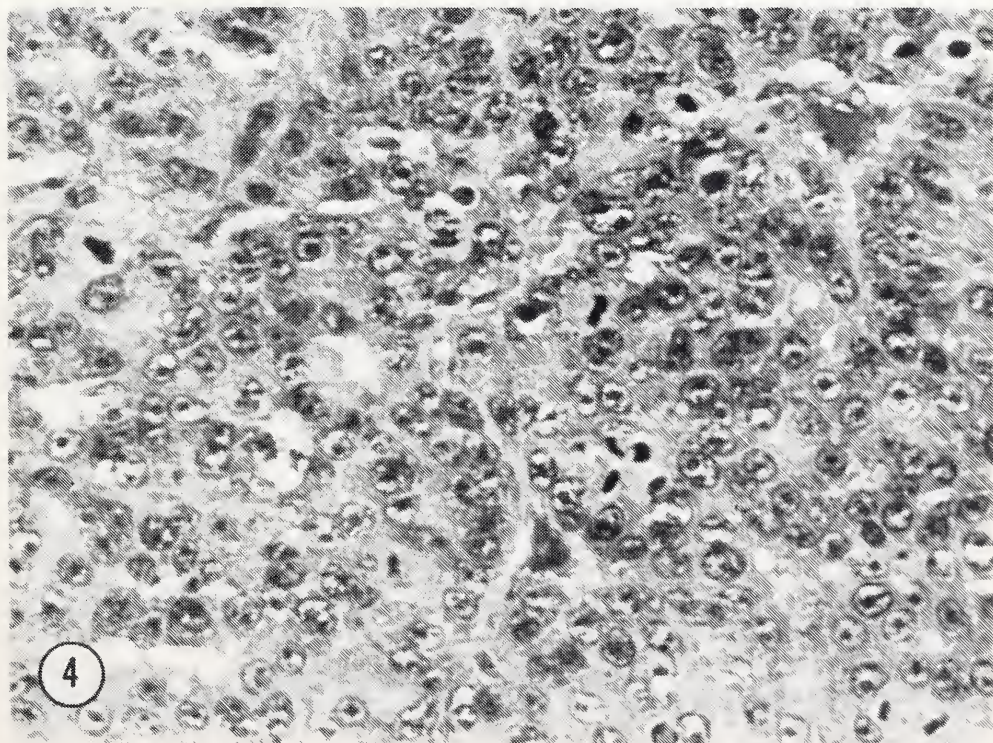


FIGURE 4. *Low magnification photomicrograph of a hepatocellular carcinoma of a rat fed a deficient amount of vitamin A. Mag. = 400X.*

tissue in the adequate group (Fig. 1) appeared less consistent in cell size with increased number of large cells with very large and pleomorphic nuclei than did those in both the excess and deficient groups (Figs. 2 and 3). Nuclei were smaller and more uniform in diameter with both dietary extremes than in the group fed adequate vitamin A. More vacuolar spaces were observed in the excess group compared to the adequate. This pattern of cavitation was reminiscent of normal sinusoid patterns and was observed in both the excess (Fig. 2) and the deficient animals (Fig. 3 and 4). PAS reactivity of cell borders and intracellular spaces appeared to be greater in the hepatomas of the excess and deficient groups compared to the adequate group as did the overall level of cytoplasmic staining.

Discussion

Very few nutritional investigations have been directed toward the prevention of cancer. Vitamin A, used as an agent to prevent epithelial cancer, is attracting increasing attention. Moon et al. (4) reported reduced incidence of mammary cancer as well as a marked reduction in the total number of cancers and benign tumors when vitamin A in the form of retinyl acetate was fed to rats following injection with the carcinogen, N-methyl-N-nitrosourea. Our findings show that either a lack of vitamin A or excessive amounts of vitamin A in the diet prevent growth of subcutaneously injected hepatocellular carcinomas. An equivalence of dietary vitamin deficiency and excess in chemoprevention is surprising but has been observed previously in studies of lysosome lability (3). The mechanism involved in prevention of tumor growth is not known.

Mitotic indexes were highest for the adequate group with those for the vitamin deficient and excess groups being the lowest which suggests that cell division was slowed by the dietary extremes. Most interesting, the pattern of tissue organization of the hepatomas of the animals fed either vitamin A excess or deficient diets was very different from that of the original poorly-differentiated hepatomas transplanted. This poorly-differentiated morphology with attendant loss of tissue organization persisted throughout the study in those animals fed diets containing adequate amounts of vitamin A. However, in the dietary extremes, in those showing tumor establishment and growth a pattern of cavitation was observed reminiscent of normal sinusoid patterns. These observations suggest strongly a return to a more normal, liver-like stage of differentiation in the few tumors that persisted in the two animal groups representing dietary extremes.

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CHEMISTRY

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Abstracts

Determination of Phenol Equivalence by Conductometric Titration. R. LEWIS VAN ATTA and ROBERT E. VAN ATTA, Department of Chemistry, Ball State University, Muncie, Indiana 47306.——Certain household disinfectants, such as Lysol, contain various substituted phenols as active ingredients. The “phenol equivalence” of these solutions may be determined conveniently by conductometric titration with concentrated lithium hydroxide, similarly standardized against liquefied phenol. Titration of at least 300 ml of diluted disinfectant or standard solution with 0.5-ml increments of 2.5 *N* titrant, using a conductivity dip cell ($K = 1.0$) and a conventional conductance bridge to monitor changes in solution conductance, yields titration curves permitting rapid, accurate location of end-points at the intersection of straight lines.

Gas Chromatographic Determination of Methyl Salicylate in Rubbing Alcohol by Standard Addition. ROBERT E. VAN ATTA and R. LEWIS VAN ATTA, Department of Chemistry, Ball State University, Muncie, Indiana 47306.——Certain commercial rubbing alcohol preparations contain 1-3 volume per cent of oil of wintergreen, added due to its pleasant odor and penetrating properties. These preparations may be conveniently analyzed for methyl salicylate content by gas chromatography with a carbowax column at oven temperatures of 175-200°C. The ester peak is symmetrical and cleanly resolved, appearing well after elution of the major components isopropyl alcohol and water. The peak height, measured from the extrapolated background base-line, is proportional to concentration. The analysis is rapid and accurate, either by graphical evaluation or appropriate calculation from unknown and standard addition peak heights.

Spectrophotometric Determination of Caffeine in Cola Drinks. ROBERT E. VAN ATTA, Department of Chemistry, Ball State University, Muncie, Indiana 47306.——Caffeine may be quantitatively determined in 5-ml aliquots of cola-containing soft drinks, made basic with 20% Na_2CO_3 . The caffeine is extracted with two 20-ml portions of dichloromethane and measured spectrophotometrically at 277 nm, λ_{max} for the ultraviolet absorption of caffeine in that solvent. Identical extractions and analyses of suitable volumes of 0.40 mg per ml standard caffeine solution yield a linear calibration curve up to 2 mg of caffeine per ml. Benzoic acid, present in the aqueous layer if the soft drink contains sodium benzoate, is not extracted with the caffeine.

Turbidimetric Determination of Lead in Pottery Extract. ROBERT E. VAN ATTA, Department of Chemistry, Ball State University, Muncie, Indiana 47306.——Lead, extracted from pottery by 4% acetic acid, may be quickly and accurately determined by precipitation as lead chromate. The precipitate is formed in a medium containing a glycerol-ethanol mixture as conditioning agent. The analysis is completed by turbidimetric measurement of the suspension, contained in 18 x 150 mm glass cuvettes, at 510 nm in a Spectronic 20 spectrophotometer or the equivalent. The composition of the extract is determined by reference to an appropriate calibration plot, apparent %T vs. $\mu\text{g. Pb per ml.}$ If the suspension is carefully prepared, mixed, and timed prior to measurement, the calibration plot is linear up to 15 $\mu\text{g per ml.}$

Chemical Analysis of the Quality of Surface Water of the Wabash River at Terre Haute, Indiana. JOSEPH R. SIEFKER and LAWRENCE W. FRENCH, Department of Chemistry, Indiana State University, Terre Haute, Indiana 47809.——The purpose of this study was to record certain qualitative chemical characteristics of water samples taken from the Wabash River, and to determine quantitatively the amounts of calcium, chloride, dissolved oxygen, dissolved solids, fluoride, iron, lead, magnesium, manganese, mercury, potassium, sodium, total solids, turbidity, and zinc in the samples. Quantitative chemical data were determined for more than 4,500 separate analyses of approximately 175 water samples. Water samples were collected over a one-year period, one to six times per month, at seven sites, including a site at Coal Creek and the Terre Haute Wastewater Treatment Plant, and five sites along the Wabash River.

Reaction of Silicic Acid with Fluoride. EUGENE SCHWARTZ, Chemistry Department, DePauw University, Greencastle, Indiana 46135; R. H. BUSEY and R. E. MESMER, Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830.——Potentiometric titrations were used to determine the products formed when fluoride was reacted with monomeric silicic acid, Si(OH)_4 , in acid solutions. The free fluoride ion concentration was monitored with a fluoride electrode and the pH with a quinhydrone electrode. Measurements were made in both perchlorate and chloride media at 0°, 25°, and 60° C. Data were analyzed and results are reported in terms of $\bar{n}(\text{H})$ and $\bar{n}(\text{F})$, the average number of hydrogen ions and of fluoride ions reacted per silicic acid molecule. The predominant product is SiF_6^{2-} . The equilibrium constant for the reaction

$$\text{Si(OH)}_4 + 4\text{H}^+ + 6\text{F}^- = \text{SiF}_6^{2-} + 4\text{H}_2\text{O}$$

is about 10^{30} at 25° C in a chloride medium. The behavior of silicic acid toward fluoride is in contrast to that of boric acid where the formation of the fully-fluoridated product BF_4^- from the precursor $\text{BF}_3(\text{OH})^-$ is kinetically hindered.

Substitution Reactions of Tungsten(O) Complexes. TROY L. BALLARD, JOHN A. MOSBO, and BRUCE N. STORHOFF, Department of Chemistry, Ball State University, Muncie, Indiana 47306.——Reactions of $\text{W(CO)}_4(\text{TMPA})$ ($\text{TMPA} = \text{N, N, N', N'-tetramethyl-1,3-diaminopropane}$) with monodentate phosphine and phosphite ligands with cone angles between 105° and 180° have been investigated. The conditions required to completely displace the chelating diamine have been determined and spectroscopic procedures developed to determine the relative amounts of *cis* and *trans* isomers formed. For example, the reaction of a large excess of diphenylmethoxy-phosphine or

diphenylmethylphosphine (cone angles of 136° and 132° , respectively) with $W(CO)_4(TMPA)$ yields *cis* complexes.

The potential for using the *cis-trans* ratio of the products as an indicator of ligand size will be discussed.

Studies of 7-Hydroxychromones. GERALDINE M. HUITINK, Department of Chemistry, Indiana University at South Bend, South Bend, Indiana 46615.——A series of 7-hydroxychromones substituted at positions -2 and -3 with methyl and phenyl groups has been studied. One member of the series, 7-hydroxy-2,3-dimethylchromone, exhibits properties that make it suitable for use as the fluorescent moiety in metallofluorescent indicators.

Chelation of Dialcohols with the NMR Shift Reagent $Eu(fod)_3$. T. L. KRUGER, J. A. MOSBO and R. PRATT, Ball State University, Muncie, Indiana 47306.——The interactions of difunctional molecules with NMR shift reagents has been studied using dialcohols and tris (1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedione) europium(III) ($Eu(fod)_3$). Equilibrium constants of complex formation were obtained from plots of chemical shifts vs. ratios of shift reagent to substrate concentrations. Chelation vs. monodentate coordination was determined from these data and from chemical shift changes induced by competition from a monofunctional alcohol. The effects of chelate ring size, conformational changes and steric interactions upon chelate formation were observed.

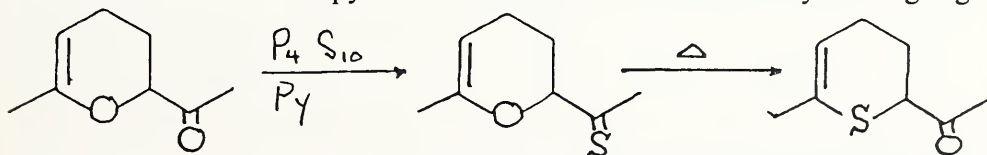
Non-Metal Pi-Face Bonding of Cyclobutadiene. K. B. LIPKOWITZ, Department of Chemistry, Indiana University-Purdue University at Indianapolis, 1201 East 38th Street, Indianapolis, Indiana 46227.——Because the gas phase lifetime of cyclobutadiene is on the order of milliseconds, it is not amenable to routine spectroscopic investigation. Generation of cyclobutadiene in noble gas matrices at very low temperature extends its lifetime so that spectroscopic studies can be accomplished. Typically an appropriate precursor is trapped in the matrix and photolyzed. Cyclobutadiene is generated but so are several photo by-products. A theoretical investigation of the pi-face bonding of cyclobutadiene with these by-products is presented.

Studies of Oxygen Exchange Kinetics Using ^{31}P and ^{13}C Nuclear Magnetic Resonance Spectroscopy. JOHN M. RISLEY and ROBERT L. VANETTEN, Department of Chemistry, Purdue University, West Lafayette, Indiana 47907.——The study of the kinetics of oxygen exchange reactions has usually involved a discontinuous assay of the system of interest; the procedure requires stopping the reaction at time intervals, isolating the compound(s) of interest, in some cases derivatizing the sample, and finally analyzing the derivative mass by spectroscopy. Cohn and Hu [Cohn, M. and Hu, A. (1978) *Proc. Natl. Acad. Sci. USA* 75:200-203] recently described the ^{18}O -isotope effect on the resonance signal of the ^{31}P nmr spectrum of inorganic phosphate. The ^{18}O -isotope effect on the ^{31}P nmr signal of inorganic phosphate is a 0.021 ppm upfield shift per ^{18}O atom bonded to phosphorus. The ^{18}O induced shift on ^{31}P nmr spectra has already been used in studies of enzyme-catalyzed exchange reactions involving phosphate-phosphate and phosphate(oxygen)-water systems [Bock, J. L. and Cohn, M. (1978) *J. Biol. Chem.* 253:4082-4085 and Webb, M. R., McDonald, G.

G. and Trentham, D. R. (1978) *J. Biol. Chem.* **253**:2908-2911]. The phosphate (oxygen)-water exchange catalyzed by homogeneous human prostatic acid phosphatase has been studied in our laboratory using highly enriched ^{18}O -phosphate [VanEtten, R. L. and Risley, J. M. (1978) *Proc. Natl. Acad. Sci. USA* **75**:4784-4787].

A comparable but potentially even more widely applicable observation would be an ^{18}O -isotope effect on the resonance position of carbon-13 in ^{13}C nmr spectroscopy. Jameson [Jameson, C. J. (1977) *J. Chem. Phys.* **66**:4983-4988] predicted such an upfield shift in the ^{13}C nmr spectrum of CO_2 and it should depend on the number of ^{18}O atoms bonded to the carbon atom. We have now observed such an upfield shift in the ^{13}C nmr resonance signal of both an alcohol and a carboxylic acid; the ^{18}O -isotope effect is 0.035 ppm per ^{18}O atom. These results are of particular utility in studies of carbon-oxygen exchange kinetics. We have demonstrated the value of the ^{13}C nmr shift induced by ^{18}O by measuring the rate of loss of the ^{18}O label from a sample of ^{18}O -t-butanol; the resultant kinetic data agrees with the literature value obtained by conventional mass spectrometry [Dostrovsky, I. and Klein, F. S. (1955) *J. Chem. Soc.* **1955**:791-796]. Thus the ^{18}O -isotope induced shifts of ^{31}P and ^{13}C nmr signals provide valuable tools for the study of kinetics, particularly since they provide continuous, non-destructive assays.

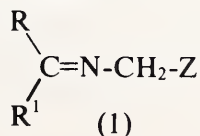
Formation and Synthetic Utility of Dihydropyrans. K. B. LIPKOWITZ and S. SCARPONE, Department of Chemistry, Indiana University-Purdue University at Indianapolis, 1201 East 38th Street, Indianapolis, Indiana 46227._____Several 2,3-dihydropyrans have been prepared via the thermally allowed 4+2 cycloaddition of α,β -unsaturated carbonyls. Since these rings are 1,5-dienes they can and do rearrange via the symmetry allowed [3,3] sigmatropic shift. Functionalization of the pyrans can then lead to new heterocyclic rings eg:



The synthesis of thiapyrans and attempted syntheses of spirocyclic natural products using this methodology is presented.

The Synthesis of Amino Acids by Phase-Transfer Reactions. MARTIN J. O'DONNELL, THOMAS M. ECKRICH, ROBIN L. POLT, GILBERT S. STATEN and KATHLEEN R. VOGLER, Department of Chemistry, Indiana University-Purdue University at Indianapolis, 1201 East 38th Street, Indianapolis, Indiana 46205._____A simple synthetic route is presented for the preparation of higher amino acids from the simplest amino acid, glycine. A key step in this sequence is the novel use of phase-transfer alkylations of glycine derivatives.

Various Schiff base derivatives (*I*) of glycine ($\text{R} = \text{aryl}$; $\text{R}^1 = \text{aryl}$, H ; $\text{Z} = \text{CO}_2\text{R}^{11}$, CN ; $\text{R}^{11} = \text{alkyl}$) are readily prepared by the condensation of a ketone or aldehyde with the appropriate amine.



Compounds *I* can be alkylated in good yields by phase-transfer alkylations. The alkylated derivatives are then readily hydrolyzed to the higher amino acids. The effect of variation in the structure of *I* and the reaction conditions on the outcome of the phase-transfer reactions will be discussed.

The Isolation of the Two Dominating Forms of Adenosine Deaminase from Frog Tissues. KENNETH S. COOK and PANG FAI MA, Department of Chemistry, Ball State University, Muncie, Indiana 47306.——Studies of adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) from mammals, reptiles, amphibians, and avians have shown three molecular forms of 200,000, 100,000, and 35,000 daltons, designated as A, B, and C forms, respectively. Ma and Fisher (1968) have shown that the tissue specific differences, with respect to adenosine deaminase in the chicken, were due to the presence of a B form enzyme in the liver which was absent in the duodenum. A C form enzyme was present in both tissues and has shown strikingly similar properties. Tissue specific differences have been reported when various frog tissues were compared. This study will attempt to isolate the two enzyme forms from frog tissue and determine a few kinetic parameters, such as the relative substrate specificities, the Michaelis constant, and the energies of activation. The results will serve as a basis to explain the differences observed in different tissues. A procedure for partial purification of the enzyme forms will also be discussed.

Effects of Ionic Strength and Buffer Composition on the Heat Stability of Bovine Erythrocyte Superoxide Dismutase. DOUGLAS B. WILLIAMS and ERIC R. JOHNSON, Department of Chemistry, Ball State University, Muncie, Indiana 47306.——Copper-zinc superoxide dismutase (E.C. 1.15.1.1) isolated from bovine erythrocytes has been found to exhibit an unusual resistance against irreversible denaturation at temperatures of 80-100°C. This heat stability is markedly dependent on ionic strength, with the enzyme showing increasing stability with decreasing ionic strength. The greatest heat stability was observed with a superoxide dismutase solution that had been exhaustively dialyzed against distilled water. The polyvalent anions, sulfate and phosphate, were found to decrease the heat stability of the enzyme to a greater extent than the monovalent chloride anion.

A Positive Ribonuclease Zymogram Technique. EMILY A. YOUNT, ROBERT C. KARN, MARGARET L. CRISP, and M. E. HODES, Department of Medical Genetics, Indiana University, Indianapolis, Indiana 46202.——A zymogram technique is presented in which the presence of ribonuclease is demonstrated by a dark band on a light background. This system has several advantages over previous methods in which the presence of ribonuclease is demonstrated by a light cleared area on a dark background of stained RNA (negative staining methods). This new technique is more sensitive, gives better resolution, takes less staining time, and is less subject to interference by nonenzymatically active protein than previous methods. This new technique involves digestion of dinucleoside monophosphate substrates of the type Pyr-p-A to produce adenosine. A series of enzymatic reactions is used to link adenosine production to reduction of a tetrazolium salt and darkening of the gel. We are presently using this technique to screen for polymorphisms in ribonucleases from human lymphocytes and granulocytes.

Practical Photometric Titrations With An Easily Constructed Reaction Cell

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Introduction

Although the general technique of photometric titration has been known for many years, activity in the field became pronounced with the accessibility of relatively inexpensive filter photometers and spectrophotometers in the decade following about 1945 (1). With the development, in 1959, of an inexpensive titration cell which provided for much larger sample volumes and stirring by circulation (2), interest was further drawn to the technique. That titration vessel, however, although relatively inexpensive and easy to construct, is rather tricky to assemble and difficult for students to employ.

A very simple, large-volume photometric titration vessel has been developed, which is readily constructed from an ordinary beaker and a test tube. It has been thoroughly tested by students who have never before performed a photometric titration and used with a variety of commonly available instruments.

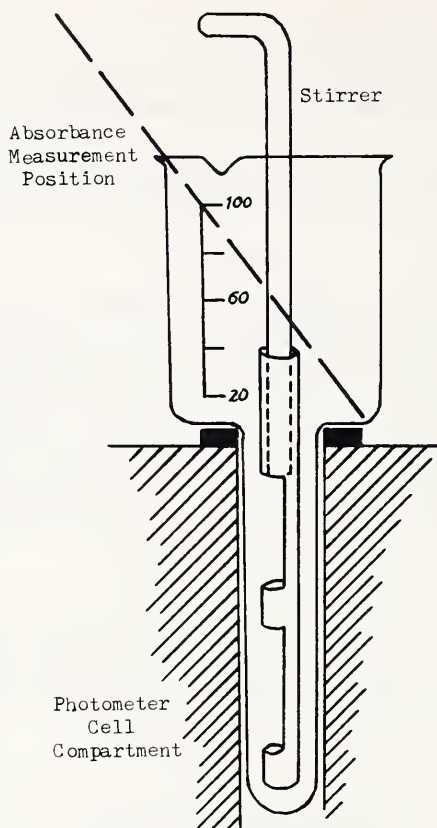
Materials and Methods

The photometric titration vessel (Fig. 1) consists of a pyrex test tube (selected so that the tube fits conveniently into the cell compartment of the instrument to be used), sealed into the bottom of a graduated pyrex beaker of the desired capacity—100 ml is a convenient size, so that titrant volumes as large as 10 ml may be used without significant change in the total solution volume. The length of the actual cell—the test tube—is determined by the working depth of the instrument cell holder, but should be kept as short as possible. The stirrer is constructed from a piece of tygon tubing of appropriate length, cut out as shown in (Fig. 1) to provide thorough solution mixing, into which a piece of glass or plastic rod of the desired length is inserted to serve as a handle.

Operational procedure is simple. After each addition of titrant, the stirrer is raised and lowered rapidly several times, in the cell, so that solution from the test tube is “pumped” up into the beaker, thus stirring the entire solution. Five to ten seconds of such stirring have been found to be adequate. The stirrer is then placed in the “measurement” position, as indicated by the dashed line in the figure, while the instrument reading is taken. Depending upon the size of the beaker used in construction of the cell, the volume of solution titrated may range from a minimum of about 5 ml to a maximum of about 125 ml.

Applications

The photometric titration vessel described may be used with almost any simple colorimeter or filter photometer having a vertical cell compartment

FIGURE 1. *Photometric Titration Vessel.*

which accepts cylindrical cells. Titration vessels have been constructed and tested with the Chemtrix Type 20 Colorimeter, Klett-Summerson Photoelectric Colorimeter, and the Markson J-7453 Colorimeter. Instruments which have an integral shutter actuated by insertion of the sample cell, such as the Bausch and Lomb Spectronic 20 Spectrophotometer, do not yield particularly good results with the cell owing to a light leakage problem—a circumstance also observed with the cell described by Rehm, *et al* (2). Inasmuch as the diameters of the cell compartments vary somewhat with the different brands of instruments, different sizes of test tube cells are generally required.

Two specific analyses have been selected for student experiments with the photometric titration cell (although many others are equally suitable). These are (a) the determination of tannins in tea or coffee by photometric titration with potassium permanganate in the presence of indigo carmine, according to the method of Loewenthal (3), and (b) the analysis of washing soda by titration of the aqueous solution with $N/2$ HCl, using methyl orange as indicator.

Procedures and Results

Determination of Tannins in Tea or Coffee.—The $N/10$ $KMnO_4$ titrant was standardized against USP grade tannic acid (0.500 g per 100 ml) by the same photometric titration procedure used in the analysis. Ten ml (pipet) of the tannic acid solution or an appropriate volume of freshly brewed unknown sample (25.0 ml of tea or 5.00 ml of coffee) were transferred to a clean cell mounted in the

instrument cell compartment. One ml (pipet) of indigo carmine indicator (0.3% in dilute H_2SO_4) and sufficient distilled water to bring the total volume up to about the 80-ml mark on the beaker were added. The titrations were carried out in the usual manner, recording sample solution absorbance at 610 nm and the volume of permanganate solution delivered after each increment of titrant was added and the solution stirred about 5-10 seconds, as previously described. Titrant was added in 1.0-ml increments through a discernible change from green to golden-yellow in the color of the sample solution in the beaker and continued until 4 or 5 increments had been added after minimum (or constant) absorbance was observed. Typical photometric titration curves for samples of tannic acid, tea, and coffee are shown in (Fig. 2).

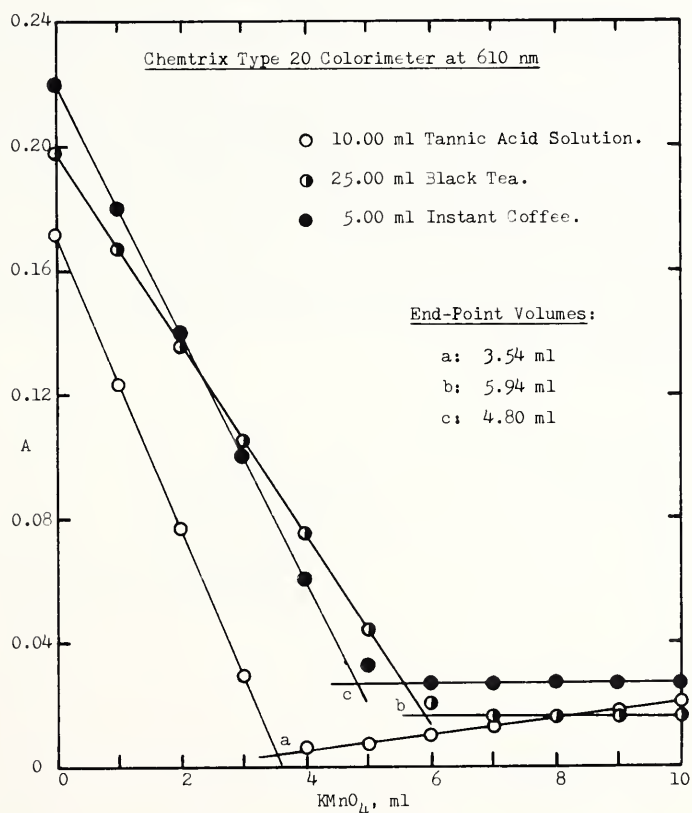


FIGURE 2. Photometric Titration Curves for the Determination of Tannins.

The results of the analyses may be widely varied, depending upon a number of experimental variables. These include time and temperature of brewing, as well as the type and brand of commercial sample analyzed. When all sample variables were fixed, it was found that photometric titrations with the cell described consistently yielded results to well within one relative percent for replicate measurements on the same system. A little imagination may produce an extensive series of interesting student individual or class experimental studies involving tea or coffee.

Analysis of Washing Soda.—This analysis involves the classical titration of sodium carbonate (commercial washing soda) with $\text{N}/2$ HCl , using methyl orange (0.1% in distilled water) as indicator. The end-point color change for this

titration has always been a difficult one for students to observe. In this case, since the HCl used was not standardized by the method to be used, an indicator blank was determined. Approximately 80 ml of distilled water were added to the clean cell mounted in the instrument cell compartment, along with about 5 drops of methyl orange indicator solution. This "blank" solution was then titrated photometrically with 0.10-ml increments of N/2 HCl (previously standardized against standard NaOH solution, using phenolphthalein indicator), until three successive increments produced no change in absorbance (Fig. 3, point A). Then a 10.0-ml aliquot of the commercial washing soda sample solution (prepared by dissolving 5.00 g of the dry commercial product in sufficient distilled water to make 250 ml) was added to the solution already in the cell as a result of the blank titration, and the titration completed, using 0.5-ml increments of acid until the first significant change in absorbance was observed (Fig. 3, point B). Then, 0.1-ml increments were added until point D was reached, after which 3 or more additional 0.5-ml increments were added to complete the titration. The titration end-point volume is the volume at straight-line intercept point C minus that at the blank titration intercept point A. The results were reproducible to within 0.5 relative per cent when the indicator blank volume was applied and the second titration curve break used as the end-point. When the HCl was standardized against primary standard Na_2CO_3 by the same photometric titration technique, the first break in the titration curve (intercept point B) was used as the end-point, leading to equivalent results.

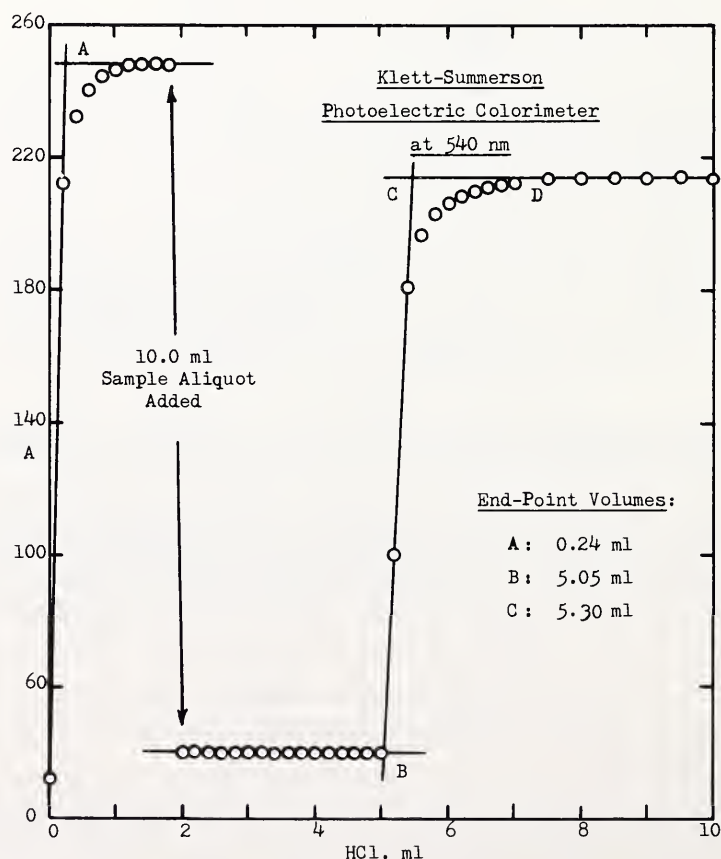


FIGURE 3. Photometric Titration of Washing Soda.

Student interest in these titrations has been high due to the fact that they may provide their own samples, the measurements are rapid and easy to complete, and the results have been exceptionally reliable. The tea/coffee experiment, in particular, has proved to be an interesting one, owing to the many variables and independent "projects" which may evolve from it.

Acknowledgment

The author wishes to acknowledge financial support for this work through a CORE grant from the Ball State University College of Sciences and Humanities.

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Potentiometric Titration Analysis of Real Samples with the Carbon Electrode

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Introduction

Although potentiometric titrations offer perhaps the widest possible applications to the analysis of all types of samples, this technique is often avoided in high schools and small colleges due to the instrumentation required and the inconvenience of the conventional electrode systems. Recently, applications of the carbon electrode to potentiometric titrations have been suggested (2), following a previous evaluation of pyrolytic graphite as an indicator electrode (1). Two analyses suitable for student use, employing a simple, inexpensive, student-constructed potentiometer (4) and home-made electrodes have been developed.

The first of these analyses is the determination of the purity of a commercial table salt (as NaCl) through potentiometric titration of the dissolved chloride with standard silver nitrate. A carbon electrode is employed in the analysis as a reference electrode, along with a piece of bare silver wire as the indicator electrode. The principal advantage of this method is that no salt bridge is required with the carbon electrode, in contrast with the usual types of reference electrodes, *e.g.*, saturated calomel or silver-silver chloride, both of which require non-chloride salt bridges as a part of the cell system for the titration of chloride with silver nitrate.

The second analysis is an acid-base titration, applied to the determination of the active component (hydrochloric acid) of certain liquid household cleaners by titration with standard sodium hydroxide; these cleaners are frequently intensely colored, so that ordinary visual indicator titrations are not feasible. The same student potentiometer is employed as in the first analysis, as is the carbon electrode. However, in this instance, the carbon electrode serves as the indicator electrode, while either a home-made or commercial saturated calomel or silver-silver chloride is suitable as the reference. The advantages of this method lie in the simplicity of the potentiometer and the fact that the carbon indicator electrode is much less expensive and somewhat more durable than the commercial glass combination electrode. This analysis may be performed quite readily with commercial equipment and apparatus.

Materials and Methods

Although any conventional student-type potentiometer may be employed for the titrations subsequently described, a great deal of student interest and experience result from the use of instruments built prior to or as part of the experiments (4). The instrument needs only to be capable of potential measurements to within 1 or 2 millivolts since, in monitoring potentiometric

titrations, changes in voltage are measured (as opposed to the absolute potentials). Consequently, the potentiometer need not be standardized prior to use, since the voltage measurements are relative. Furthermore, absolute measurements with the carbon electrode tend to drift somewhat from one titration to another, as previously reported by Miller (1).

The carbon electrode, used either as indicator or reference, may be made from a carbon rod or stick at least two inches in length. Center rods, removed from dead dry cell batteries (1) or 1/8-inch diameter lead from "kindergarten" pencils serve adequately. Whatever the source, the carbon rod must be pre-soaked in concentrated hydrochloric acid for 15 minutes or so to remove acid-insoluble impurities, washed thoroughly with distilled water, and dried prior to initial use. Electrode sensitivity is maintained if the HCl treatment is repeated after each dozen or so titrations; the electrode should be thoroughly rinsed with distilled water after each titration.

The silver indicator electrode used in the chloride analysis may be made conveniently from a small piece of fairly heavy gauge silver wire, with a 1/4-inch loop bent into the bottom. The wire should be polished with emery paper or cloth, rinsed quickly with dilute nitric acid, and thoroughly rinsed with distilled water before initial use. It should be rinsed well with distilled water after each titration.

The saturated calomel electrode used as reference in the acid-base titration may be readily constructed. Various forms of this electrode, any of which are applicable, have been described by Vogel (5) and others.

The titration vessel itself may consist conveniently of a beaker of appropriate size (150 to 250 ml) containing a magnetic stirrer bar to provide adequate stirring throughout the titrations. A standard 25- or 50-ml buret may be used to deliver the titrants.

Procedures and Results

Analysis of Table Salt.—The N/10 AgNO_3 titrant was prepared and standardized by weight (3). A 5.00-ml aliquot (pipet) of unknown sample solution (prepared by dissolution of an accurately weighed 2.0-g sample of the dry commercial table salt in sufficient distilled water to make 100 ml of solution) was transferred to the titration beaker containing the magnetic stirrer bar. Five ml (graduated cylinder) of dilute HNO_3 (to prevent precipitation of carbonate and other acid-soluble silver salts) were added, along with about 50 ml of distilled water, so that the C/Ag electrode pair was immersed in the liquid. The carbon reference electrode was connected with the positive terminal of the potentiometer and the silver indicator wire with the negative. Using a medium stirrer speed throughout the experiment, the solution was titrated with silver nitrate delivered from the buret, using volume increments as required to yield good definition of the titration curve. The solution was stirred for 5-10 seconds after each titrant addition to provide adequate stirring before voltage measurements were made.

A typical titration curve is shown in (Fig. 1A). The end-points were evaluated in the usual way for potentiometric titration curves. Results obtained

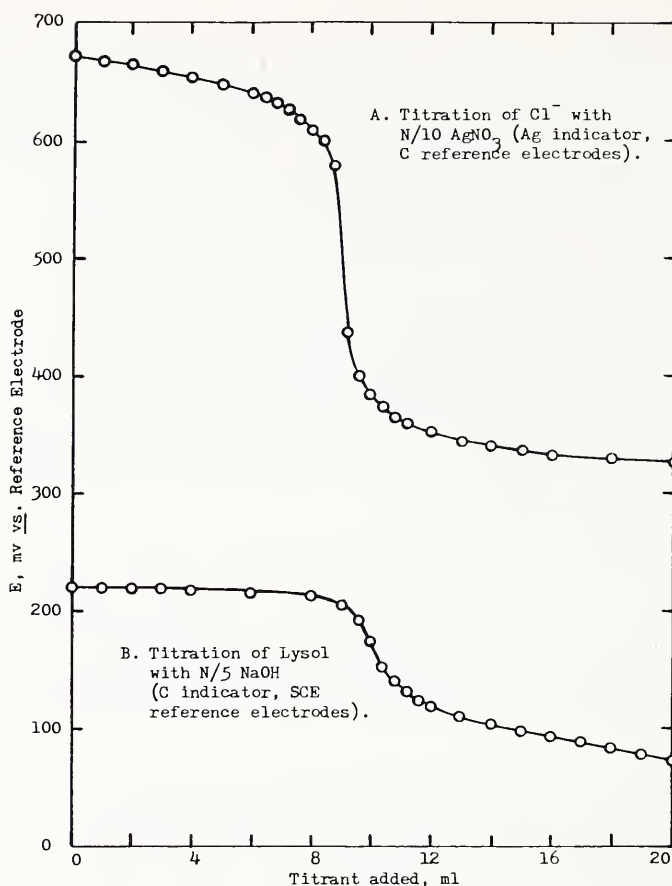


FIGURE 1. Potentiometric Titrations Involving Carbon Electrodes.

TABLE I Potentiometric Analysis of Table Salts.

Commercial Product	Sample Size g/100 ml	Found % NaCl
A	1.34	98.2
	2.48	98.1
	3.55	98.1
B	1.42	97.6
	2.56	97.8
	3.61	97.5
C	1.37	96.4
	2.34	97.0
	3.45	96.6

for three different commercial table salt samples are shown in Table I. The results are independent of sample size, as shown by the data, while replicate titrations yielded values which agreed within 0.5 relative per cent. Analyses of three 2.0-g samples of primary standard grade NaCl (99.9% pure) yielded a result of $99.9 \pm 0.2\%$.

Analysis of Household Cleaners.—The $\text{N}/5 \text{ NaOH}$ titrant was standardized against primary standard potassium acid phthalate by the conventional visual titration with phenolphthalein as indicator (3). A 1.00-ml sample (pipet) of the commercial liquid cleaner was transferred to the titration

beaker containing the magnetic stirrer bar and sufficient distilled water added so that the C/SCE electrode pair was immersed in the liquid. The saturated calomel electrode was connected with the negative terminal of the potentiometer and the carbon indicator with the positive. The titration with sodium hydroxide was performed in the same manner as the chloride titration described previously.

A typical titration curve for the neutralization titration with the carbon indicator electrode is shown in (Fig. 1B). Replicate results obtained for two commercial household cleaners are shown in Table II. Results for the same two cleaners obtained by similar titrations using a commercial pH meter and combination glass electrode were virtually identical with those shown in the table.

TABLE II *Potentiometric Analysis of Household Cleaners.*

Sample Analyzed	% HCl Present	HCl Equivalent Found, %
X	9.25	9.4, 9.3, 9.2
Y	8.50	8.6, 8.4, 8.6

Students have shown considerably more interest in these titration experiments than they have in earlier experiments involving purchased pre-analyzed samples and commercial apparatus. The instruments are less likely to be considered as magical black boxes by beginning students, and the home-made electrode systems (along with the analysis of "real" commercial samples provided by the students) are practical illustrations of the value of the techniques. Commercial product comparison and agreement with labeled package contents are interesting additional factors of this type of experiment.

Acknowledgment

The author wishes to acknowledge support for this work in the form of released load-time provided by a CORE grant from the Ball State University College of Sciences and Humanities.

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Ionization of Aqueous Mandelic Acid: Conductance and Thermodynamics

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Introduction

Mandelic acid is a water soluble, moderately weak acid. Its properties are adapted to measurement of its ionization behavior yet almost nothing about it has been reported in the literature to date. It does have some uses in analytical chemistry and in medicine. As part of an ongoing study of the thermodynamics of weak acid ionization we report here conductance measurements on mandelic acid for concentrations between about one millimolar and one-tenth millimolar for a temperature range of 10 to 75 degrees Celsius. The only other report in the literature closely related to our work is one study by Banks and Davies (1) who found mandelic acid to have a pK 3.411 at 25°C measured by conductivity.

Experimental

Solution preparation and measurement procedures were essentially the same as described earlier by Strong, Kinney, and Fischer (15). A set of four conductivity cells was used to obtain conductivities for eight different concentrations. D(-) mandelic acid (D- α -hydroxyphenylacetic acid) was purchased from Sigma Chemical Company as a high purity preparation and was used without further treatment other than drying it over phosphorus pentoxide in a vacuum dessicator at room temperature for several weeks.

Results

Molar conductivities for mandelic acid are listed in Table I for eight concentrations and fourteen temperatures. At the foot of the table molar concentrations at 25°C are listed while molarities at other temperatures were calculated by multiplying each listed concentration by the ratio of the density of water at each desired temperature to the density at 25°C. Calculations indicated that any difference between the density of water and the various solutions was less than 0.01%.

In Table II are presented the derived values of Λ_0 , $pK_a(c)$, and $pK_a(m)$ together with the standard errors calculated from the scatter of the experimental values around the calculated values. The two sets of pK_a values reflect two different standard state choices: (1) an ideal one molar standard state represented by $pK_a(c)$ which is the result of the fact that solution conductivity is a volume property and (2) an ideal one molal standard state represented by $pK_a(m)$ which is the more desirable choice for thermodynamic analysis. The conversion of $pK_a(c)$ to $pK_a(m)$ was done as proposed by Robinson and Stokes

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TABLE I *Mandelic Acid—Molar Conductivities*

Deg C.	$\Lambda_0, \text{cm}^2 \Omega^{-1} \text{mol}^{-1}$									
10	130.99	138.04	143.98	221.32	221.60	231.03	234.72	237.89		
15	143.44	151.15	157.64	242.39	242.72	253.08	257.13	260.56		
20	155.49	163.86	170.95	263.04	263.42	274.74	279.14	282.76		
25	167.15	176.22	184.28	283.27	283.68	295.97	300.70	304.68		
30	178.26	187.97	196.71	302.87	303.33	316.57	321.66	325.95		
35	188.82	199.15	208.56	321.84	322.57	336.55	342.19	346.60		
40	198.79	209.73	220.02	340.02	340.93	355.79	361.82	366.48		
45	208.15	219.69	230.77	357.45	357.76	374.27	379.91	385.61		
50	216.86	229.01	241.33	374.03	374.82	391.95	398.02	403.89		
55	224.91	237.63	251.28	389.79	390.80	408.65	415.23	421.38		
60	232.33	245.71	261.08	404.64	406.58	424.70	431.74	437.97		
65	239.08	253.20	271.53	418.60	421.89	439.77	447.42	453.65		
70	245.30	260.30	281.41	431.63	436.43	453.87	462.33	468.49		
75	250.86	266.84	292.06	443.77	451.05	467.11	476.70	482.45		
$C \times 10^3 \text{ @ } 25^\circ \text{C}$	1.1705	1.0094	0.8971	0.18103	0.18055	0.14354	0.13278	0.12219		

TABLE II *Mandelic Acid: Limiting Conductance and Ionization Constant*

Temp. °C	Λ_0 $\text{cm}^2\Omega^{-1}\text{mol}^{-1}$	St. Err.	$\text{pK}_a(\text{c})$	St. Err. $\times 10^4$	$\text{pK}_a(\text{m})$	$\sigma\%$
10	296.14	0.34	3.3976	23	3.3975	0.06
15	324.50	0.37	3.3986	22	3.3982	0.06
20	352.66	0.39	3.4020	22	3.4012	0.06
25	380.65	0.42	3.4072	22	3.4059	0.06
30	408.34	0.45	3.4147	22	3.4128	0.06
35	435.92	0.61	3.4247	27	3.4221	0.07
40	462.79	0.70	3.4354	29	3.4320	0.08
45	488.40	0.35	3.4456	14	3.4414	0.04
50	514.36	0.48	3.4591	18	3.4539	0.05
55	539.63	0.65	3.4736	23	3.4673	0.06
60	564.63	1.27	3.4893	42	3.4819	0.11
65	589.06	2.31	3.5057	72	3.4972	0.18
70	612.54	3.58	3.5216	106	3.5118	0.26
75	635.92	5.71	3.5389	160	3.5278	0.39

(13) using $\text{pK}_a(\text{m}) = \text{pK}_a(\text{c}) + \log (\text{H}_2\text{O density})$. The righthand-most column of Table II lists $\sigma\%$, the standard deviation of the experimental Λ values from those calculated and presented as a percentage of Λ zero at each temperature.

Table II shows that the precision of the measurements ranged between 0.05% and 0.1% up to 60°C but deteriorated markedly above 60°C. The decrease in precision seemed to be a consequence of slight leaks of solution along the seal between the platinum electrode leads and the glass of the electrode chamber. These leakage difficulties differed from cell to cell and increased with rise in temperature.

In Fig. 1 $\text{pK}_a(\text{m})$ is plotted as a function of temperature in the lower part of the figure while the Walden product, $\eta_0\Lambda_0$, for the mandelate ion is plotted in the upper part of the figure. Vertical bars indicate the calculated standard error at each temperature. For comparison, data for benzoic acid (15) are included in the figure. The continuous curve drawn for mandelic acid has been calculated to give the best fit when each datum is weighted by $1/(\text{standard error})^2$.

Analysis of the conductivity-concentration relation at each temperature was made with a conductance function recently proposed by Fuoss (3) in which

$$\Lambda = [1 - \alpha(1 - \gamma)] [\Lambda_0(1 - \Delta X/X) - \Delta \Lambda_c]$$

where ΔX is the relaxation effect correction generated by the external field X , $\Delta \Lambda_c$ is the electrophoretic effect correction, $1 - \gamma$ is the fraction of the ions that are paired while α represents the fraction of the paired ions that are in contact. This 1978 function differs from the one proposed by Fuoss in 1975 (4) and used in the treatment of the benzoic acid data (15) primarily in the way the dissociation constant enters into the model. In this new model the division between ions and unionized molecules includes the effect of diffusion of the ions out of the Gurney sphere to become free ions that contribute to the flow of charge. However, for mandelic acid the two functions give Λ_0 and pK values that differ by a negligible amount compared to the scatter, 0.05% of Λ_0 and less than 0.001 for pK .

There is just one measurement of pK and Λ_0 reported in the literature by Banks and Davies (1) who found $pK_a(c) = 3.411$ by conductivity at 24.92°C . We have corrected their data to 25.00°C and done a recalculation by the same procedure used for our data and find $pK_a(c) = 3.4062$ and $pK_a(m) = 3.4051$ with a standard error of 0.0010. This is in good agreement with our $pK_a(m) = 3.4059$. Their Λ_0 recalculated is $378.03\text{ cm}^2\Omega^{-1}\text{mol}^{-1}$ with a standard error of 0.26 which is not in terribly close agreement with our $\Lambda_0 = 380.65$. It does seem likely that Λ_0 for mandelic acid would not be much different from that found for benzoic acid where $\Lambda_0 = 381.72$ at 25°C .

Walden products for the mandelate ion at the various temperatures were calculated. For this calculation the limiting conductance of the hydrogen ion was subtracted from the limiting conductance of mandelic acid to give the limiting anion conductance which when multiplied by the viscosity of water gave the Walden product at each temperature for the mandelate ion. These are assembled in Table III and displayed in Fig. 1 where the vertical bars show the calculated errors derived from the standard error in Λ_0 values. The continuous curve is derived from a cubic polynomial in the temperature that fits the

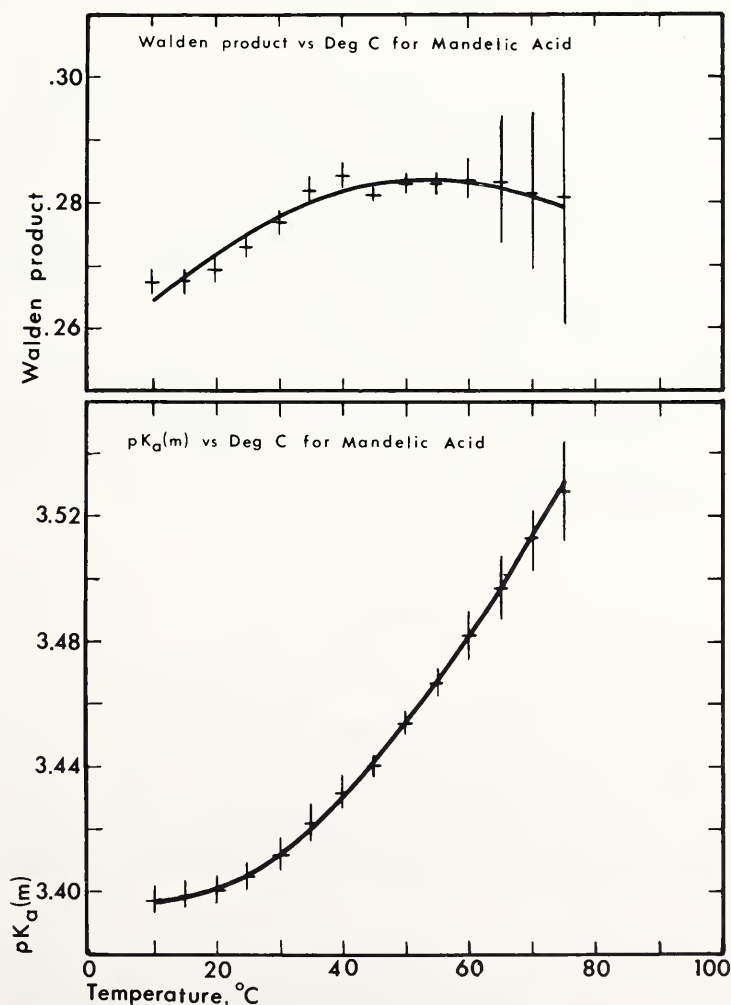


FIGURE 1. Walden Product, $\eta_0\lambda_0$, and $pK_a(m)$ for mandelic acid. Vertical bar shows standard statistical error assigned to each datum.

TABLE III *Mandelate Ion: Limiting Conductance and Walden Product*

Temp. °C	$\Lambda_0(\text{obs})$ $\text{cm}^2\Omega^{-1}\text{mol}^{-1}$	Dev. ^a	λ_0^- $\text{cm}^2\Omega^{-1}\text{mol}^{-1}$	Walden Product $\eta_0\lambda_0^-$	Dev. ^b
10	296.14	-0.19	20.32	0.265	0.004
15	324.50	0.08	23.59	0.268	0.004
20	352.66	0.23	27.12	0.272	0.004
25	380.65	0.19	30.86	0.275	0.004
30	408.34	0.07	34.79	0.277	0.004
35	435.92	-0.34	38.89	0.280	0.004
40	462.79	-0.46	43.12	0.281	0.005
45	488.40	0.23	47.46	0.283	0.002
50	514.36	0.10	51.88	0.284	0.003
55	539.63	0.18	56.35	0.284	0.003
60	564.63	0.01	60.84	0.284	0.006
65	589.06	-0.13	65.32	0.283	0.010
70	612.54	0.13	69.77	0.282	0.015
75	635.92	-0.09	74.16	0.280	0.022

^a $\Lambda_0(\text{calc}) - \Lambda_0(\text{obs})$ where $\Lambda_0(\text{calc}) = 237.76 + 5.8747T - 5.3181 \times 10^{-3}T^2 - 2.9910 \times 10^{-5}T^3$

^bUncertainty in Walden product produced by applying all the standard error for Λ_0 listed in Table II to λ_0^- .

observed Λ_0 with an average deviation of 0.17 conductance units. The hydrogen ion conductances used in these calculations are derived from an equation given by Harned and Owen (6) based on the conductivity data for hydrochloric acid of Owen and Sweeton (11). However, these data extend only from 5° to 65° C. To include values beyond 65° C a Λ_0 value at 100° C cited by Quist and Marshall (12) was combined with the transport numbers of Macdonald and Owen (9) to calculate a $\lambda_0^-(\text{H}^+)$ at 100° C of $648 \text{ cm}^2\Omega^{-1}\text{mol}^{-1}$.

With increasing temperature $\eta_0\lambda_0^-$ for the mandelate ion increases at least up to 55° C. For higher temperatures the uncertainties in the data are large enough to preclude any decision as to the pattern although the data suggest that the Walden product goes through a maximum near 55° C. The observed increase is about 7% of $\eta_0\lambda_0^-$ at 10° C.

Discussion

From the variation of $\text{pK}_a(\text{m})$ with temperature it is possible to derive the thermodynamic parameters ΔH° , ΔS° , ΔC_p , and their variation with temperature. Whether this is practical hinges on the number of data points, the temperature range, and the precision of the data. The most careful considerations of a suitable calculation strategy have been made by Clarke and Glew (2) and by Ives and Moseley (8). Their conclusion is that the proper function to describe the temperature pK relation is $\text{pK} = A + B/T + C\log T + DT + \dots$ where terms after the logarithm term form a power series in T.

For the mandelic acid data the precision and temperature range of the data are sufficiently limited that only the first three terms of the fitting equation are clearly justified. Therefore the Ives and Moseley sigma plot method was used. The resulting equations are:

$$pK_a(m) = -37.6882 + 1738.97/T + 14.2508 \log T$$

$$\Delta G^\circ = 7956.9 + 65.206T \log T - 172.45T$$

$$\Delta H^\circ = 7956.9 - 28.319T$$

$$\Delta S^\circ = 144.13 - 65.206 \log T$$

$$\Delta C_p^\circ = -28.3 \text{ cal/mole-deg}$$

The energy units in these equations are calories per mole and the temperatures are Kelvin. The fitting is such that calculated pK values agree with observed values with an average deviation of less than 0.02% or about 1 unit in the third digit to the right of the decimal in the pK values.

TABLE IV *Thermodynamic Parameters for Four Acids at 25° C*

	ACETIC ACID ^a	BENZOIC ACID	GLYCOLIC ACID ^b	MANDELIC ACID
$pK_a(m)$	4.756	4.1985	3.831	3.4059
ΔG° , cal/mol	6487.	5728.	5226.	4648.
ΔH° , cal/mol	8.7	61.	163.	-486.
$-\Delta H^\circ/T$, cal/mol-deg	-0.029	-0.20	-0.547	1.63
ΔS° , cal/mol-deg	-21.7	-19.0	-17.0	-17.2
ΔC_p° , cal/mol-deg	-29.3	-38.6	-39.9	-28.3

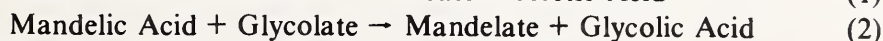
^aHarned and Ehlers (5) data recalculated to give $pK_a(m) = -38.1023 + 1909.45/T + 14.7317 \log T$.

^bNims (10) data recalculated to give $pK_a(m) = -54.5736 + 2630.22/T + 20.0379 \log T$.

The thermodynamic parameters at 25° C are given in Table IV. Some useful comparisons can be made between mandelic acid and related substances such as acetic acid (5), glycolic acid (10), and benzoic acid (15). For the first two we recalculated the $pK(T)$ function by the sigma plot method. However, neither the acetic acid nor the glycolic acid data extend over a wide enough temperature range nor are represented by enough data points to be entirely satisfactory for calculating thermodynamic parameters from curve fitting.

Comparison of mandelic acid with these other three related acids can best be made on an entropy plot as described by Strong and Halliwell (14) Fig. 2 is such a plot with ΔS° , the standard entropy change for the system, on the abscissa and $-\Delta H^\circ/T$, the standard entropy change for the environment, on the ordinate. Diagonal dotted lines indicate constant pK values since $R \ln 10 pK = -\Delta G^\circ/T = -\Delta H^\circ/T + \Delta S^\circ$. The circles locate the experimental temperatures. Dashed lines connect points at the same temperature for the different acids.

The striking relation among the selected acids revealed by Fig. 2 is that acetic, benzoic, and glycolic acids ionize with little difference in $-\Delta H^\circ/T$ anywhere within the temperature range studied. By contrast mandelic and glycolic acids differ in ionization mainly in $-\Delta H^\circ/T$ while differing little in ΔS° . It is convenient to compare two contrasting acid-base reactions in relation to the data



These will be referred to as symmetric reactions.

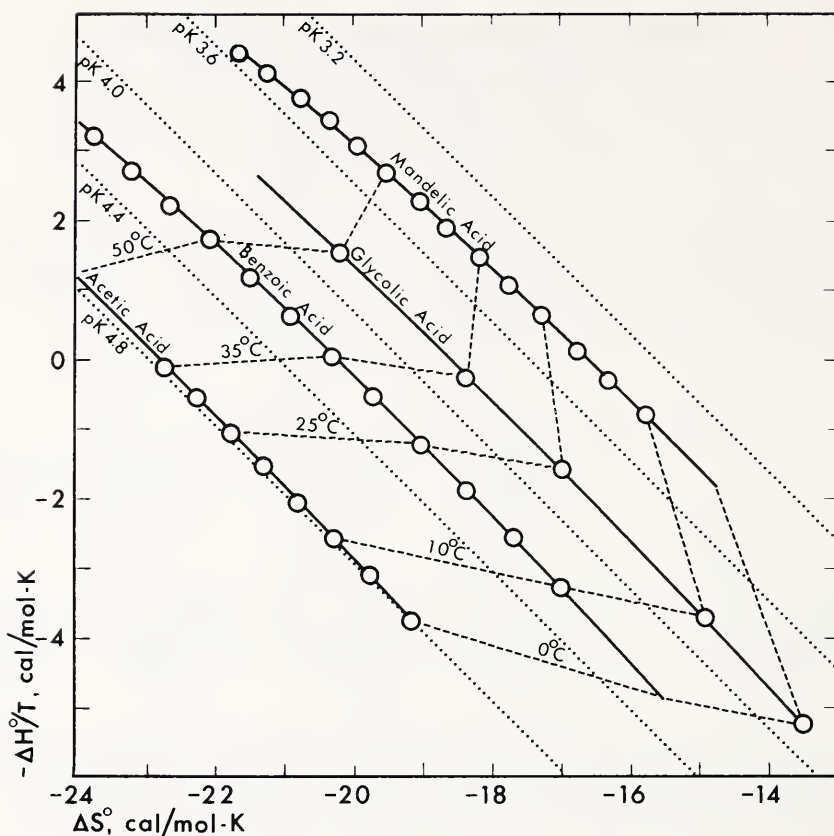


FIGURE 2. Entropy plot for the ionization of four acids in water. The continuous solid curves are calculated from the equations that best fit $pK_a(m)$ vs. T . Each dotted line indicates a constant pK ranging from 3.2 on the right to 4.8 on the left. Circles correspond to the experimental temperatures and the dashed lines connect points of the same temperature for the four acids.

Hepler and Wooley (7) have described a way of interpreting such symmetric reactions. Enthalpy and entropy changes are divided into an internal part which refers to the interaction of the charged ions only and an external part which refers to the interaction of the acid and base with solvent. Thus

$$\Delta H^\circ = \Delta H_i + \Delta H_e$$

$$\Delta S^\circ = \Delta S_i + \Delta S_e$$

Generally for symmetric reactions $\Delta S_i = 0$. Hepler and Wooley suggest that

$$\Delta H_e = \beta \Delta S_e + \gamma \Delta H_i$$

where it is usually found that $\beta \approx T$ and γ is a constant related to solvent and temperature. For reaction (1) $\Delta H^\circ = 0$ therefore $\Delta H_i = -\Delta H_e$. The question of whether ΔH_e or ΔH_i is negative can be approached by considering ΔC_p° which is negative ($-39 + 29 = -10$ cal/mol-deg) for reaction equation (1). A negative ΔC_p° implies a decrease in molecular motion which points to increased hydration for the benzoate-acetic acid pair compared to their conjugates. It is then reasonable to assume that increased hydration is accompanied by decreased enthalpy or $\Delta H_e < 0$. Therefore $\Delta H_i > 0$ so that the attraction by acetate ion for a proton is less than by benzoate ion. It must then be that it is increasing hydration which drives the reaction by raising the entropy of the system. The entropy rises because water molecules initially tied into the water structure gain some freedom of motion on transfer to the acid-ion system.

In the mandelic acid-glycolate system $\Delta C_p^\circ = -28 + 40 = +12$ cal/mol-deg. In line with the Hepler-Wooley analysis ΔH_e is proportional to ΔH_i . Since $\Delta C_p^\circ > 0$ it is reasonable to assume that hydration decreases as the reaction proceeds and that $\Delta H_e > 0$. Therefore we conclude that $\Delta H_i < 0$ and sufficiently large to yield $\Delta G^\circ < 0$.

In summary benzoic acid transfers a proton to acetate ion as a consequence of increased hydration. Mandelic acid transfers a proton to the more electrically attractive glycolate ion.

Of the four acids discussed here Walden products for a range of temperatures are available only for benzoate and mandelate ions thus far. Strong, Kinney, and Fischer concluded that the Walden product for the benzoate ion did not change significantly ($< 4\%$) with increasing temperature. It now appears that the mandelate ion has a Walden product that increases some with increasing temperature. This can be taken to indicate that the mobility of the mandelate ion increases with temperature by an amount that is greater than that determined solely by the increasing fluidity of the solvent. Therefore the hydrodynamic volume of the mandelate ion decreases as the temperature increases which implies that solvation decreases with increasing temperature. Furthermore this implies that solvation of the glycolate ion also decreases with increasing temperature but no conductance data are available to verify such a conclusion.

Further work is underway to improve the precision and extend the temperature range of the pK data.

Conclusion

Mandelic acid has been found to have a $pK_a(m)$ of 3.406 at 25°C and to decrease slightly in acidity as the temperature rises. Compared to glycolic acid with a pK of 3.831 the greater acidity of mandelic acid is due to a smaller attraction for positive charge by the mandelate ion compared to the glycolate ion which favors ionization of mandelic acid. The Walden product for the mandelate ion increases with increasing temperature up to about 55°C but beyond that temperature it may decrease slightly although the precision of the present data is inadequate to be sure about the decrease.

Curiously the substitution of a phenyl group seems to have different consequences when acetic and benzoic acids are compared than when glycolic and mandelic acids are compared. The implications of this difference are not yet clear.

Acknowledgments

We are indebted to Dr. Raymond Fuoss for the computer program that processes conductivity data and to Dr. Thomas Copeland for assistance in refining various of our computer programs as well as in the interpretation of the statistical analyses. One of us (DD) was supported by a grant from the E.I. duPont deNemours Education Fund. The Earlham College Professional Development Fund provided support for this work as well.

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Cleavage of Modified Adenosine Nucleotides by Two Phosphatases with Differing pH Optima

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Introduction

Various ADP analogs have been investigated for effectiveness as inducers of platelet aggregation *in vitro* (7, 12). This information is used to assess requirements of the platelet ADP receptor for certain nucleotide structural features (4). Since platelet rich plasma may contain some phosphatase activities (2), the short-range susceptibility of the ADP analogs to phosphatase cleavage must be investigated initially to rule out possible secondary effects with the platelet suspension system from nucleotide hydrolysis products. Some novel information found about the substrate specificities of the two model phosphatases used for this purpose is reported in this present investigation.

Materials and Methods

The ADP analogs were obtained from Sigma Chemical Company, St. Louis, Missouri, or from P-L Biochemicals, Inc., Milwaukee, Wisconsin as chromatographically homogeneous crystalline powders. The two enzymes were purchased from Sigma Chemical Company as acid phosphatase (P-3752) and as alkaline phosphatase (P-3877). The nucleotide analogs were dissolved in the buffer appropriate for the incubation with a given enzyme present at the concentration of 1.0 mg/ml. Alkaline phosphatase was dissolved in 0.1M glycine—NaOH buffer, pH 10.0 at 1.0 mg/ml concentration while acid phosphatase was dissolved in 0.1M disodium citrate, pH 5.0 at the same concentration. Aliquots of enzyme (0.1 ml) were added to 1.0 ml of substrate in the appropriate buffer, mixed and incubated at 25°C. Aliquots (30 μ l) of reaction mixture were spotted at one hour intervals on cellulose MN-300 (Sigma Chemical Company) thin layer plates (15 g powder slurried in 90 ml of distilled water, coated on glass plates in 0.25 mm. layers, and air dried overnight) and developed immediately in a solvent mixture consisting of saturated ammonium sulfate (80 ml): 1.0M sodium acetate (18 ml): isopropanol (2 ml) (9). Compounds were visualized by ultraviolet illumination or by exposure to iodine vapor and were identified by R_f comparison with standards chromatographed on the same plate.

Results

All parent compounds were well resolved from possible hydrolysis products in this chromatography solvent system and easily detected at indicated concentrations. The use of cellulose thin layer plates for the superior resolution of nucleoside, nucleotide, or carbohydrate mixtures has been documented previously (11). The four compounds mentioned in this report were shown to be

stable in the pH 3 - pH 10 range at room temperature (25°C) for periods up to four hours; this suggests that all were suitable from this stand point to be used in platelet aggregation studies. Upon prolonged incubation in 0.155M NaCl, pH 6.8, at 25°C, the following relative stabilities (in decreasing order) were demonstrated by thin layer chromatographic analysis: meth ADP >> Me ADP, ϵ ADP, ϵ AMP > AMP > ADP.

TABLE I *Relative Susceptibility of Adenosine Nucleotides and Analogs to Potato Acid Phosphatase (3.1.3.2)*

AMP > ϵ AMP
ADP > ϵ ADP
MeADP: No change in 5 hours
MethADP: No change in 5 hours

The relative susceptibilities of the modified adenosine nucleotides to acid phosphatase attack are shown in comparison to the corresponding naturally occurring adenosine nucleotides in Table I. It can be seen that the additional bulky ring present in the etheno compounds (ϵ AMP and ϵ ADP) causes some impedence of enzyme mediated phosphate cleavage. These etheno compounds may very well be the largest purine-like substrates used with acid phosphatase so far. Both MeADP and methADP are resistant to acid phosphatase attack, showing that this degree of pentose substitution is not tolerated by the enzyme and that this P-C-P linkage is resistant to enzymatic cleavage as is expected.

TABLE II *Relative Susceptibility of Adenosine Nucleotides and Analogs to Calf Intestine Alkaline Phosphatase (3.1.3.1)*

AMP; ϵ AMP: Similar Susceptibilities
MeADP > ϵ ADP
2ADP $\leftrightarrow$ AMP + ATP
MethADP: No change in 5 hours

Table II shows the comparative susceptibility of these adenosine derivatives to alkaline phosphatase hydrolysis. As with acid phosphatase, the bulky etheno compounds are tolerated, and the P-C-P linkage is not cleaved by this enzyme. The pentose derivative, MeADP, is hydrolyzed by alkaline phosphatase, indicating that some steric accomodations are available in the active site of this enzyme, or that other binding regions are of greater importance in determining enzyme-analog interaction. Although AMP is observed more rapidly in the reaction mixture when ADP is used as principal substrate, ATP is also ultimately detected, suggesting transphosphorylation has occured: 2 ADP $\leftrightarrow$ AMP + ATP.

Discussion

It is not too surprising that ϵ AMP and ϵ ADP serve as substrates for both of the present phosphatases since Secrist *et. al.* have shown that ϵ ATP could be used as a phosphate, pyrophosphate or adenylate donor, or as an allosteric

effector in selected ATP requiring enzyme systems (10). The additional bulky ring is well tolerated by the present enzymes and it is seen that there is no requirement for a free amino group on the purine ring for hydrogen bonding interactions with either of the active sites of the two enzymes. Changes in location of cationic charge and pK are also not problematic in present enzyme—analog interactions; other regions in these etheno nucleotide molecules may be of greater importance in this interaction. Concerning bulky substrate analogs, Ikawa *et. al.* (5) hydrolyzed several phenyl substrates with acid phosphatase and Cathala *et. al.* (1) reported that even bulky naphthyl substrates are well tolerated by alkaline phosphatase.

This report states that MeADP is cleaved by alkaline phosphatase, but not by acid phosphatase. This compound, and one other pentose variant, adenine arabinofuranoside-5'-diphosphate, were shown to be poor substitutes for ADP at the platelet ADP receptor in platelet aggregation studies by Ragatz *et. al.* (8). Lesser substrate specificity for variations in sugar moieties of other analogs with alkaline phosphatase has been previously reported (1, 3).

The P-C-P linkage found in methADP is not cleaved by either phosphatase. This is to be expected from other enzymatic studies reported by Myers *et. al.* (6). Acidic or basic hydrolysis of this compound is only accomplished upon prolonged treatment at 100°C (6). Transphosphorylation does not occur with any of the analogs in the presence of alkaline phosphatase, but does occur with ADP as substrate, as reported previously by Georgatsos for the placental enzyme (3).

These three kinds of analogs have been shown to be stable over a broad pH range and in the presence of two phosphatases analogous to enzymatic activities which may be encountered in human plasma. Thus, the present compounds are deemed appropriately stable for platelet aggregation studies. The present investigation has also shown that an impressively bulky ring system can be accommodated by the active site regions of two unique phosphatases, that pentose conformation is important in limiting the interactions of some analogs with nucleotide-utilizing enzymes, and that P-C-P bonds remain stable under diverse hydrolytic circumstances.

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Isozymes of Human Urine Ribonuclease Demonstrated by Isoelectric Focusing¹

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Abstract

Ribonuclease (RNase) from human urine demonstrates a multiplicity of bands of activity following isoelectric focusing on thin layer polyacrylamide gel (IFPAG) and a negative-staining zymogram technique. Most of the activity focuses at a pH of 3.5-4.0 on the pH gradient, but numerous other bands of activity are located throughout the isoelectric spectrum, extending to about pH 9.5 at the cathode. The urine RNase banding patterns on IFPAG have exhibited uncontrollable variation in different urine specimens from the same individual and in the same specimen stored for different periods of time. Treatment of urine with neuraminidase reduces the complex and variable banding pattern to a single major band which focuses at a pH of about 9.5 (on a broad range "pH 3.5-10" gradient). Thus sialic acid residues apparently account for a series of transtional isozymes, the most acidic of which has an isoelectric point of up to 6 pH units lower than the desialydated enzyme. A two-stage method for alkaline range IFPAG, previously developed in this laboratory, with supplementation of the commercial ampholytes with arginine to extend the pH gradient in the alklaine range, has demonstrated 2 bands of RNase activity in neuraminidase-treated urine which focus at pH 9.5-10.

Introduction

The existence of charge isomers (isozymes) of human urine ribonuclease (RNase) was first demonstrated in 1958 using ion exchange chromatography (9). Subsequently, charge heterogeneity of urine RNase was observed in several investigations employing ion exchange chromatography (4, 8, 19, 20, 21, 28, 42) and electrophoresis (16, 33). Since this previous work did not include a search for variation in isozyme pattern among individuals, it is not known whether this enzyme(s) is polymorphic. In an effort to detect a genetic polymorphism in RNase from human urine, we have employed isoelectric focusing in polyacrylamide gel (IFPAG), a technique which appears to offer greater resolving power than traditional electrophoretic techniques (31). this communication describes the complex isozyme pattern of human urine RNase demonstrated by IFPAG, its variability in specimens from a single individual, and the need to remove sialic acid residues from this glycoprotein before attempting to evaluate a potential polymorphism.

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Materials and Methods

Urine from a single individual was collected over thymol in a plastic cup and either frozen or held at 4° for about 2 hours before processing. The fluid was dialyzed in Spectrapor 1 membrane tubing (M. W. cutoff: 6,000-8,000) against deionized water for 24 hours at 4°. The dialyzed urine was lyophilized and either stored at -20° or reconstituted for immediate analysis with deionized water at one-twentieth the original volume.

One concentrated urine specimen was treated with neuraminidase (Sigma, Type VI, from *Clostridium perfringens*) at 0.1 u/ml (0.05 mg/ml) in 0.1 M sodium acetate buffer, pH 5.0, and dialyzed against this buffer for 24 hours at room temperature and against deionized water for 24 hours at 4°. Controls for the neuraminidase treatment included a aliquot of concentrated urine treated with buffer only and dialyzed as above, and another aliquot stored at 4° for the 48 hour period.

Equipment, materials, and the general procedure for IFPAG in our laboratory have been described elsewhere (36). The composition of the thin layer polyacrylamide gels (1.5 mm thick and 19 cm long) used in this study was as follows: 3.64% acrylamide (T = 4%), 0.36% N,N'-methlenebisacrylamide (C = 9%), 20% glycerol, 0.8% Ampholine pH 3.5-10, 0.1% Ampholine pH 2.5-4, 0.1% Ampholine pH 9-11, 0.05% L-arginine (free base), 0.00005% riboflavin. The electrolyte solutions were 1.0% Ampholine pH 2.5-4:20% glycerol for the anode and 0.2% L-arginine: 20% glycerol for the cathode. Samples were applied as described previously (36) at 10 cm from the cathodic end of the gel. The electrofocusing runs were started at 800 V in a constant power mode so that the voltage increased to 2000 V prior to the end of the run, which lasted for 8 hours.

The zymogram method for detecting RNase activity in polyacrylamide gels was a modification of the techniques of Wolf (41) and Wilson (40). Following IFPAG the gel was soaked twice (15 minutes each time) in a solution of 0.1 M sodium phosphate buffer, pH 6.0 or 8.0, with agitation at room temperature, and again in 0.05 M buffer. The gel was incubated in a solution of 1.0 mg/ml RNA (Calbiochem, purified from *Torula*, B grade, M. W. 20,000-30,000) in 0.05 M sodium phosphate buffer, pH 6.0 or 8.0, at 37° for 1 hour. Subsequently, the solution was removed, the gel was rinsed in 0.05 M buffer for 2 minutes, and the incubation was continued (after removal of buffer) in a humidified chamber at 37° for an additional hour. The gel was fixed in 5% acetic acid at 4° overnight, stained in a solution of 0.2% toluidine blue in 0.5% acetic acid over a one-day period.

Results

Examples of the isozyme patterns of RNase from human urine following IFPAG in a broad range pH gradient are illustrated in (Fig. 1). With this negative-staining zymogram technique, bands of RNase activity appear as clear areas against a darkly stained RNA background. Numerous bands of activity are located throughout the isoelectric spectrum. In any given sample, most of the activity focuses near the anode at a pH of 3.5-4.0 on the pH gradient. The remainder of the banding pattern shows much variation with different urine

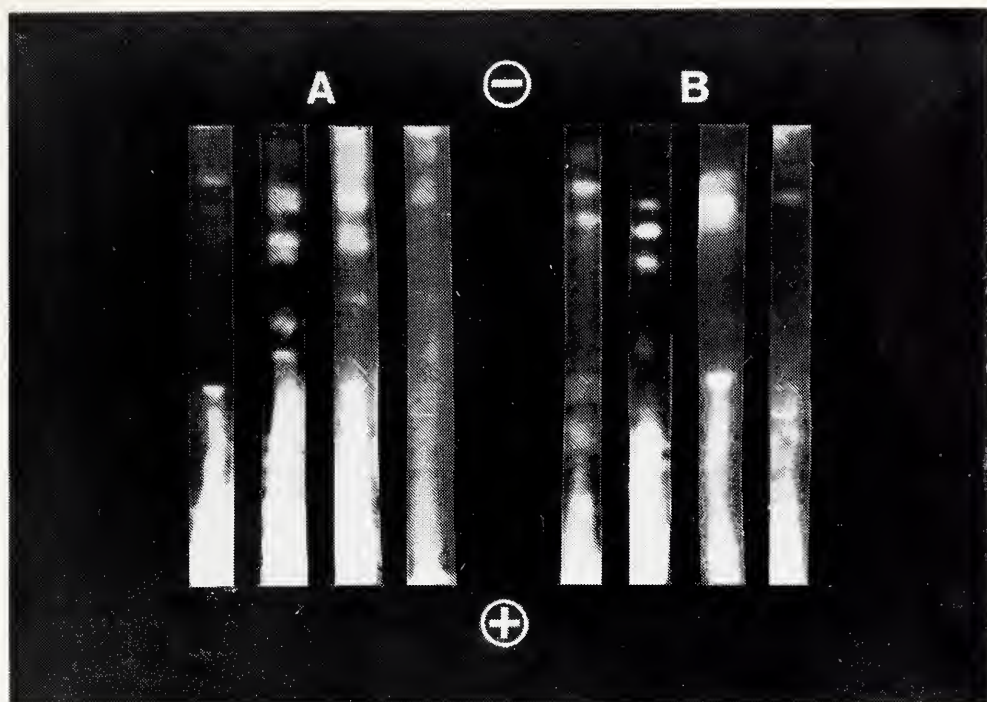


FIGURE 1. Examples of the IFPAG patterns of the isozymes of human urine RNase, with equilibration and incubation at pH 6.0 (A) and at pH 8.0 (B). The pH gradient extends from about pH 3.0 at the anode to about pH 9.7 at the cathode. For each strip 40 μ l of 20-fold concentrated urine was applied. This amount results in excessive activity in the acidic region, but enables the development of minor bands in the alkaline region. The first three strips in each group are patterns from the same specimen stored (lyophilized) for various periods of time, and the last strip in each group is the pattern from a different specimen.

specimens from the same individual and with the same specimen stored for different periods of time. Variation is especially evident in the alkaline region. This variation appears to be uncontrollable and can not be attributed to the initial freezing of a urine specimen or the length of storage of a lyophilized sample.

Treatment of urine with neuraminidase, which removes sialic acid residues from glycoproteins, has a dramatic effect, as illustrated in (Fig. 2). The complex and variable banding pattern is reduced to a single band which focuses at a pH of about 9.5.

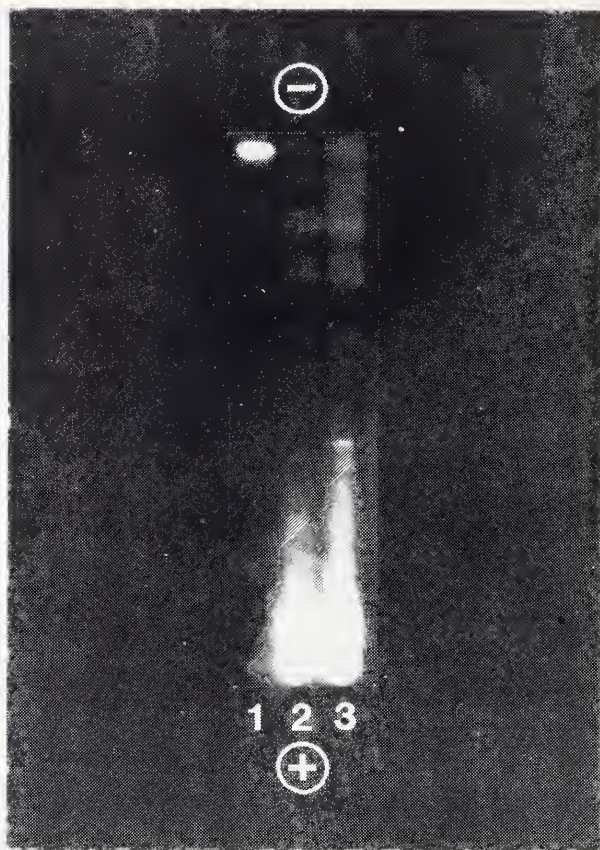


FIGURE 2. IFPAG patterns of the isozymes of human urine RNase, with and without treatment of the urine with neuraminidase. Equilibration and incubation were at pH 6.0. The pH gradient is the same as in (Fig. 1). 1): 40 μ l of 20-fold concentrated urine sample treated with neuraminidase (diluted 1:2 for treatment). 2): 40 μ l of 20-fold concentrated urine sample treated with buffer only (diluted 1:2 for treatment). 3): 20 μ l of 20-fold concentrated urine sample stored at 4° (not diluted).

Discussion

The present demonstration of the isozymes of human urine RNase by IFPAG has been accomplished by certain modifications of the method which deserve mention. Since the urine RNase isozymes can focus at very low or very high pH's, the commonly-used "pH 3.5-10" Ampholine must be supplemented with Ampholines of pH 2.5-4 and pH 9-11 in order to extend the gradient at the extremes. To enable the retention of the most alkaline band on the gel, it is supplemented with arginine (pI 10.7), which also comprises the cathodic electrolyte. Following IFPAG, the gel must be extensively equilibrated with a buffered solution to overcome the buffering capacity of the Ampholines and establish a constant pH throughout the gel. To accomplish this, the gel was soaked in buffer for a much longer period than that recommended by Wilson (40). Also, incubation of the gel after removal of the substrate solution was found to be necessary to detect minor bands of activity. As have other investigators (30, 39, 40), we have determined that the negative-staining zymogram may be non-specific for RNase, since concentrated zones of a protein (e.g., albumin in serum) may yield a false-positive band. This possibility is excluded from our analysis of urine, since there are no detectable protein bands after the application of urine at this level of total urinary protein.

The results of treatment of urine with neuraminidase indicate that sialic acid residues apparently account for a series of transitional isozymes of RNase, the most acidic of which has an isoelectric point (pI) of up to 6 pH units lower than the desialydated enzyme. Many hydrolytic enzymes from animal tissues are known to be glycoproteins and contain sialic acid (7, 10, 11, 22, 23, 26). Treatment of these various enzymes with neuraminidase results in shifts from acidic to basic forms, increases in pI's, and reduction or abolition of heterogeneity (7, 10, 22, 23, 26). The reported changes in pI's following neuraminidase treatment of various hydrolytic enzymes vary from about 0.5 to 2.5 pH units (7, 22, 23, 26). Thus the pI increase we have observed for desialydated human urine RNase appears to be unusual. While there are no reports of a human RNase containing bound sialic acid, Reddi (29) suggested that the urine enzyme was a glycoprotein on the basis of a positive stain with periodic acid-Schiff reagent. With respect to nonhuman RNases, one report demonstrated that neuraminidase treatment of the soluble fraction from rat kidney lysosomes altered the electrophoretic mobility of RNase bands to more basic forms (10).

Other reports of the pI's of human RNases are of interest. RNase purified from human urine has been reported to have a pI of 3.5 (28) and 4.1 (29), as determined by isoelectric focusing in a sucrose density gradient. These values agree well with the major area of activity on our IFPAG gels when using untreated urine. RNase purified from human pancreas had a pI of 10.3, as determined by traditional electrophoretic mobility measurements (38). Also, RNases purified from human serum and leukocytes reportedly had pI's above 11.0, as determined by the density gradient focusing technique (2, 3). These basic pI values appear similar to the pI of the desialydated urine RNase.

As mentioned previously, charge heterogeneity of human urine RNase has been observed in several investigations. Human RNase from other fluids or tissues also appeared heterogeneous. Ion exchange chromatography demonstrated heterogeneity of RNase in serum (1, 2, 3, 4, 5, 6, 14, 32, 34), leukocytes (18), pancreas (38), and epidermis (17). Electrophoretic methods demonstrated heterogeneity of RNase in serum (5, 6, 14, 30), leukocytes (13, 27, 30), erythrocytes (30), pancreatic juice (37), liver (30), spleen (12, 13), kidney (30), heart (30), testes (30), and epidermis (15). The degree of heterogeneity observed in these investigations ranged from 2 to 8 peaks or zones of activity. Since most tissue RNases reportedly are present in urine (4, 25), further investigations concerning the sites of origin, relationship and significance of the various human RNases should include the effect of neuraminidase treatment. In addition, the present study has demonstrated that treatment of urine with neuraminidase is requisite for determining possible heterogeneity and genetic polymorphism inherent to the urine RNase protein.

Preliminary results using a two-stage method for alkaline range IFPAG to enhance the resolution, similar to our method described elsewhere (36), indicate that neuraminidase-treated urine shows 2 bands of RNase activity which focus at pH 9.5-10. In this regard, it is of interest that other investigators have determined the existence of two distinct classes of human RNases, both of which occur in urine (4, 24, 25).

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ECOLOGY

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Abstracts

The Indiana Natural Heritage Program. JOHN FEINGOLD and JOHN BACONE, Department of Natural Resources, 612 State Office Building, Indianapolis, Indiana 46204, and MARION T. JACKSON, Indiana State University, Terre Haute, Indiana 47809, and The Nature Conservancy, 1800 North Kent Street, Arlington, Virginia 22209.——Indiana became the 11th state to initiate a State Natural Heritage Program when Governor Bowen signed a contract between the Department of Natural Resources and The Nature Conservancy on May 19, 1978. The program will be administered under the direction of John Feingold, Division of Outdoor Recreation, Department of Natural Resources, in close cooperation with the Division of Nature Preserves and The Nature Conservancy.

The Heritage Program is an intensive and ongoing statewide inventory of the significant individual components that comprise Indiana's natural diversity. The natural features, or elements, on which information is collected includes plant and animal species of special interest (eg. endangered, threatened, vulnerable), fragile or critical habitat types, and other uncommon natural phenomenon. Data regarding these target elements are collected for storage in a variety of inter-connected and cross-reference filing systems, including a set of U.S.G.S. 7½ minute topographic maps and manual and computer files. Major uses are expected to be research, natural area preservation, and environmental impact assessment oriented.

The organization, operation and utility of Indiana's Heritage Program are discussed.

Flora and Plant Communities of Spicer Lake Nature Preserve, St. Joseph County, Indiana. VICTOR L. RIEMENSCHNEIDER, Department of Biological Sciences, Indiana University at South Bend, South Bend, Indiana 46615.——Spicer Lake, a pothole lake located on the Valparaiso moraine in the extreme northwest corner of St. Joseph County, Indiana is an excellent example of lake succession. The approximately 1.4 hectares of open water is surrounded by a yellow pond lily-swamp loose-strife community that is floating on its lakeward side. The shrub zone behind the pond lilies rapidly blends into the shrub layer of the surrounding swamp forest. The shrub zone contains swamp rose, buttonbush, red osier, winterberry and blueberry. Spicebush becomes the dominate shrub as the shrub zone merges with the swamp forest shrub layer. Cinnamon and royal ferns are also important in this zone and goldthread, relatively rare in Indiana, is a common herb. The swamp forest is

dominated by red maple, 67% of the 22.3 m²/ha basal area, with slippery elm an important associate. The herb layer is dominated by a number of species including wild sarsaparilla and canada mayflower. Several other small communities add to the floristic diversity of this 16 hectare natural area.

A Preliminary Annotated Bibliography of Aquatic Studies. Published in the Proceedings of the Indiana Academy of Science, 1891-1935 and H. E. McREYNOLDS, U.S. Forest Service, Bedford, Indiana 47421._____The author reviews biological studies of an aquatic nature published in the Proceedings of the Indiana Academy of Science during the 1891-1935 period. A brief resumé of the contents of these papers is provided. This should aid the reader in deciding whether or not he needed to review the particular paper in its entirety. The author plans to abstract later Proceedings papers (1936-1977) in the future.

Prediction and Evaluation of Aquatic Communities Using Environmental Data. RONALD A. HELLENTHAL, Biology Department, University of Notre Dame, Notre Dame, Indiana 46556._____The prediction of model aquatic communities for specific habitats has long been a critical need in environmental biology. This need is most acute in the evaluation of compliance monitoring programs and in the assessment of the potential impact of introduced toxic substances. A computer based system called EROPT can predict assemblages of organisms using environmental requirements and pollution tolerance data. It can be used to scrutinize biological data from ecological studies to insure that habitat conditions are consistent with the organisms reported, and can show ecological similarities among related taxa, determine the level of taxonomic identification required for the indication of specific ecological conditions, and isolate erroneous environmental information associated with aquatic organisms. The EROPT system consists of computer programs for the capture, storage, and retrieval of environmental information on aquatic organisms. Programs are written in Fortran and the system has been implemented on an IBM 370/168 computer at the University of Notre Dame. Environmental requirements and pollution tolerance data for the system were obtained by digitizing environmental monitoring reports of the U.S. Environmental Protection Agency.

The Indiana Lake Classification Program: The Condition of Indiana Lakes and a Comparison of Trophic State Indices. W. HERBERT SENFT, BYRON G. TORKE and HAROLD BONHOMME._____During the years 1973-1976, over 450 Indiana lakes and reservoirs were sampled for a variety of water quality parameters. These parameters were used to define a broad trophic classification scheme for Indiana lakes. This scheme was evaluated by comparing it to Carlson's trophic state index. It was found that Carlson's index was generally a much poorer indicator of water quality than has been assumed. These results are used to assess the current trophic state and water quality of Indiana lakes.

Impact of Heavy Metals on the Growth of Yellow Perch (*Perca flavescens*) as Measured by RNA-DNA Ratios. PAUL K. KEARNS, G. J. ATCHISON, and R. J. VETTER, Bionucleonics Department, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907._____Yellow perch (*Perca flavescens*) collected from industrially contaminated Palestine Lake were analyzed for cadmium and zinc content by atomic absorption

spectrophotometry. Ribonucleic acid—phosphorus, deoxyribonucleic acid—phosphorus content of fish and RNA-DNA ratios were determined in an effort to compare growth of fish from the contaminated area of the lake to the growth of fish from the uncontaminated area of the lake.

Of three sampling dates, one revealed significant differences in growth of fish as monitored by length and weight measurements, condition and RNA-DNA ratios between sites. Linear regression analysis of length and weight relationship of fish from the two sites indicated that fish from the uncontaminated area have heavier weights to a given length than fish from the contaminated area of the lake. As body burdens of cadmium increased in yellow perch, condition and RNA-DNA ratio values decreased. Whole body concentrations of zinc had no relationship to condition or RNA-DNA ratios.

Pigeon-Borne People Diseases. WALTER WEBER, Pesticide Consultant, 36 West Roberts Road, Indianapolis, Indiana 46217.——Feral pigeons (*Columbia livia*) are not harmless birds. They have the potential for transmitting over 40 diseases to man and animals. Eight examples follow. Candidiasis primarily affects the mouth, intestines and urogenital tract, especially the vagina. The itching, pain and discharge caused by this fungus is significant enough to warrant the elimination of pigeons which spread the disease. Chlamydiosis affects the respiratory system. Over half of the pigeon population is infected. Cryptococcosis frequently affects the brain covering. It is acquired by inhalation. The yeast is carried in the intestinal tract of pigeons and deposited in their feces. One Indianapolis victim of cryptococcosis spent 81 days in the hospital. Encephalitis is transmitted through bites of infected mosquitoes after they have bitten a host. Pigeons, English sparrows and house finches are the main reservoir. *Salmonella tyhimurium*, often the cause of food poisoning is found in about 2% of pigeon feces. Toxoplasmosis has an affinity for brain tissues. When 80 pigeons were trapped on the Nation's Capitol, 10 revealed evidence of Toxoplasma infection. Yersiniosis is associated with pigeons. The symptoms are indistinguishable from appendicitis. The disease was responsible for 14 unnecessary appendectomies in 1976. Bird mites (*Dermanysus gallinae*) were responsible for a case of pruritis in a N. Carolina hospital. The mites originated in a pigeon nest in the ventilator. Additional pigeon-borne diseases include American trypanosomiasis aspergillosis, blastomycosis, coccidiosis, erysipeloid, histoplasmosis, listeriosis, Newcastle, paratyphoid, pasteurellosis, pox, pullorium, trichomoniasis, and tuberculosis.

Ecological Notes on *Robackia demeijerei* (DIPTERA: CHIRONOMIDAE)—An Example of A Well Adapted Sand Dwelling Larval, Midge. RICHARD WHITMAN, Indiana State University, Evansville, Indiana 47112.——*Robackia demeijerei* (Krusemann) has been found throughout Palearctic and Nearctic regions and is an excellent example of a highly specialized psammorheobiont adapted for a carnivorous life in unstable stream sands. Gut contents of this midge indicates that its principle food item must be *Aeolosoma* sp. and/or *Rhynchoscolex simplex*. Population dynamics of this midge was investigated in an east Texas sandy stream during 1976. Monthly mean density ranged from 4200 to 9800 org/m³ with an overall mean of 7700 org/m³. Seasonal density did not differ in most areas sampled. Mean monthly standing crops ranged from

12.9 to 49.2 $\mu\text{g}/\text{m}^3$. A good long normal relationship existed between mean weight by instar and head capsule length. *R. demeijerei* preferred sandy areas just upstream from pools and within the center of riffles while pools and margins had lower densities. *R. demeijerei* is found most abundantly at a 5-10 cm sand depth.

A Preliminary Study of the Occurrence of Broken Back Syndrome in Northern Cavefish (*Amblyopsis spelaea* Dekay) at Spring Mill State Park, Mitchell, Indiana. JAMES H. KEITH, Director, Division of Nature Preserves, Indiana Department of Natural Resources, and LOIS MITTINO GRAY, Naturalist, Spring Mill State Park, Indiana Department of Natural Resources._____In July 1976, the staff of Spring Mill State Park noted that several individuals of Northern Cavefish in Upper Twin Cave were apparently injured. Subsequent examination of the fish by a biologist from the U.S. Fish & Wildlife Service Laboratory in Genoa, Wisconsin indicated that the fish probably suffered from "Broken Back Syndrome", a condition in which vertebrae crack and extensive hemorrhaging results. This syndrome occurs under a variety of circumstances including physical trauma, vitamin C deficiency, and exposure to pesticides. Some preliminary work by the Indiana State Board of Health revealed the presence of toxaphene in the stream water of Upper Twin Cave. Results published by other investigators suggest that exposure of fish to toxaphene and related compounds alters the ratio of collagen: mineral in the skeleton. Bones become brittle and crack easily, resulting in a broken back and associated hemorrhaging.

In the summer of 1977, a similar condition was found. Using funds provided by Indiana's Division of Fish and Wildlife, it was decided to systematically survey the blind fish population and measure the pesticide content (toxaphene and dieldrin) of the stream water on a regular basis. In the summer of 1978, water samples were taken from Upper Twin Cave and analyzed for the presence of pesticides. Samples were taken bi-weekly and during major floods. Five visual censuses of the fish population were implemented to note any changes in the incidence of the syndrome over a two year period. Individual fish were also collected for chemical analysis.

A comparison of recent census data from Upper Twin Cave with data taken from the same cave by Poulson (unpublished) in 1958 and 1964 does not demonstrate an adverse impact on the population at this time. However, the water analyses point to the need for further investigation and monitoring of this system. The potential impact of continued pesticide contamination of the cave water will be discussed along with the need for further study.

The Ecology of the Troglobitic Beelte *Pseudanophthalmus tenuis stricticollis*: Recruitment and Fecundity Data. JERRY H. KEITH, Director, Division of Nature Preserves, Indiana Department of Natural Resources._____ *Pseudanophthalmus tenuis* inhabits caves in south-central Indiana. In Murray Spring Cave near Paoli, Indiana, the subspecies *Pseudanophthalmus tenuis stricticollis* is the numerically dominant predator in the arthropod community inhabiting the streamside mudbanks. In 1973 and 1974, a study of the mudbank community of Murray Spring Cave was undertaken. Because of its size, and numbers present in the cave, *P.t. stricticollis* was intensively studied.

Mark-recapture studies, censuses and field observation indicate that *P.t. stricticollis* has a seasonal life cycle that is strongly influenced by cave flooding. This differs from other organisms studied in that community, since large ($>5\text{mm}$), mobile organisms tended to have constant numbers over time and small ($<2\text{mm}$) organisms had numbers which varied in response to flooding. *P.t. stricticollis* is large and mobile, and should fit into the former category but fits, instead, into the latter category. Reasons for these differences are discussed.

The Effect of Water Level Fluctuation on Primary Productivity in Lake Monroe. WILLIAM Y. B. CHANG, Department of Biology, Indiana University, Bloomington, Indiana 47401.——Changes in the amount of discharge from the watershed causes significant fluctuation of water level in Lake Monroe. During the dry season when the discharge is decreased significantly and evaporation is intensified, the water level decreases very noticeably, resulting in a significant increase in cations and calcium concentration. This increase in cations and calcium concentration can change the water chemistry and coprecipitate available phosphate ions in the lake. In the case of soft water mesotrophic Lake Monroe, where phosphate is limiting, the effect of coprecipitation becomes strong when it is compounded with the increase of pH attributed to intensive photosynthetic activities. This effect can act to the disadvantage of phytoplankton, and may function as an indirect density-dependent population control.

Branch Abscission and Leaf Fall Relationships in a Mature White Oak. WILLIAM R. CHANEY, Department of Forestry and Natural Resources, Purdue University, West Lafayette, Indiana 47907.——Litter fall from a mature *Quercus alba* L. growing in Martell Forest was sampled weekly from August through January for five years. Litter was separated into abscised twigs, leaves attached to abscised twigs, abscised leaves, and acorns. Two phases of leaf fall were evident. The first was characterized by abscission of twigs with leaves still attached whereas the second phase was characterized by shedding of individual leaves. The peak of twig abscission with attached leaves preceded the peak of individual leaf fall by 10 to 14 days. Approximately 25% of the leaves shed in early autumn were attached to abscised twigs whereas approximately 10% of the total leaf litter was lost from the tree while attached to twigs.

Composition and Homogeneity within an Aspen-White Pine Successional System in Kalkaska County, Michigan. EDWIN R. SQUIERS,* DAVID N. ALLAN, and CARRIE, L. YOUNG, Department of Biology, Taylor University, Upland, Indiana 46989.——The composition and homogeneity of an even aged aspen-white pine successional system was studied at The Taylor University Biological Station, Big Twin Lake, Kalkaska County, Michigan. Density, frequency, and basal area were recorded for each tree species in a series of one hundred 100m^2 circular quadrats chosen at random on ten subsites within the system. Importance values and several dominance/diversity indices were calculated to describe each subsite. Similarity matrices followed by cluster analysis were then used to assess the homogeneity of the total system. Present composition within the system can be explained in terms of species life history, reproductive strategy, and the pattern of past disturbances.

Chemical and Biological Effects of Natural Annual Dieback of *Myriophyllum spicatum* Upon the Littoral Waters of a Large Soft Water Reservoir. DIXON H. LANDERS, Indiana University, Bloomington, Indiana 47401.——Three polyethylene enclosures (dia: 2 m) were placed in the littoral zone (depth: 1.5 → 2.0 m) of the upper basin of a soft water reservoir during late summer 1977 to determine the effects of natural macrophyte dieback upon the water chemistry and phytoplankton. Enclosures were located in a monospecific stand of the Eurasian watermilfoil (*Myriophyllum spicatum*). Total phosphorus and reactive phosphorus concentrations were significantly higher in the water of the enclosures with rooted and unrooted decaying plant material than in the enclosure without macrophyte tissue. A reactive phosphorus pulse ($5 \rightarrow 9 \mu\text{g l}^{-1}$) was detected as above-sediment decay occurred in the enclosure containing rooted plants. The pulse dissipated as the plants sank to the sediment surface. No reactive phosphorus was detected in the enclosure lacking macrophyte tissue. A high peak of $\text{NH}_4\text{-N}$ appeared coincident with the reactive phosphorus pulse in the enclosure containing rooted macrophytes. Phytoplankton responses to this natural nutrient addition are discussed.

Log Decomposition in Donaldson's Woods, Spring Mill State Park. P. C. MACMILLAN, Hanover College, Hanover, Indiana 47243.——Stem maps made by Lindsey and Schmelz, et al., of the trees on 20 acres of Donaldson's Woods were used to identify the logs found and to estimate their residence time (RT) on the forest floor. I collected 162 samples of wood from 125 different logs representing 10 genera. Logs were assigned to decay classes 1 to 5 on the basis of physical appearance and assigned to RT classes of 1, 8, 18 & 23 years based on information from the stem maps. The use of these two classification schemes will be discussed. Total log frequency on the forest floor was 6.25 logs/ha; log input rate was calculated at 0.22 logs/yr/ha for ca. 28 years. Only oaks (*Quercus alba*, *Q. rubra* & *Q. velutina*) were found in sufficient abundance (64 of 125 logs) to yield good statistical data. The decay rate of oak logs was calculated to be $k = -0.094$ using the logarithmic decay model $Y = a + k \cdot \ln t$ ($r^2 = .99$). Other physical parameters of the logs will be discussed. This study was supported in part by a grant from the I.A.S.

Development of a Proposed List of Endangered and Threatened Vertebrate Animals for Indiana

H. E. McREYNOLDS, U. S. Forest Service

**JOHN O. WHITAKER, JR., Indiana State University, Terre Haute, Indiana 47809
and**

JAMES R. GAMMON, DePauw University, Greencastle, Indiana 46135

The first warnings of the overexploitation of our lands, our waters and our wildlife came many years ago—some in the previous century. In the 1850's and 1860's, Henry Thoreau and George Perkins Marsh authored cautionary essays on what we were doing to our lands and waters. Later in the 19th Century, John Muir castigated Congress for its failure to protect the incomparably beautiful lands of the west—our Yosemite, our Grand Canyons, our Sierra Peaks. In the first half of this century, Aldo Leopold, Stephen Mather, Robert Sterling Yard, and Robert Marshall issued admonitory blasts against our exploitive ways.

Not, however, until mid-century did these early warnings convince a significant portion of our population that these dangers were real. The conservationists' victory in the Echo Dam Battle of the 1950's convinced them to drive on to a formalized protection—the Wilderness Act of 1964. With our special lands supposedly inviolate, our special animals were the next to receive our protective attention. The Endangered Species Act of 1966 was passed. Clean air and clean water acts were also enacted. Later amendments to the Endangered Species Act extended protection to marine mammals, foreign mammals (prohibited importation), and endangered plants. Presently we are seeking help for non-game animals.

The enactment of a law is a simplistic precursor to the tougher task of implementing it. Each piece of restrictive legislation must necessarily result in lessening or eroding of some previously accepted or implied right or freedom. The man in the middle is the land manager who must see that the dictates of the law are being implemented. He must deal, on the one hand, with that public which feels that its previous freedoms have (unjustly, of course!) been taken from it. On the other side, he is being pushed to apply the advocates' interpretation of the legislation stringently—perhaps even beyond what appears to have been congressional intent.

The Endangered Species Act compounded the usual problems by applying to animals of extremely low populational densities. Indeed, this was the primary requisite for their selection—animals that were generally the least evident and often those animals about which we knew the least. These animals were so rare that even the most astute authorities could not tell the land manager whether or not the species of concern occurred on the lands or in the waters for which the manager was responsible.

On public lands, Congress had dictated that protective management be instituted. These management practices may, however, be expensive. The managers' quandary: To install protective management practices unquestioningly, or to wait until there are proven losses of these rare animals on

the lands under his responsibility. The first approach exposes the manager to possible embarrassment when later experience shows that his expensive protection of a species was installed on lands or waters where the species did not occur. The second approach subjects the manager to charges of insensitivity and dereliction of his responsibilities.

A compromise approach would involve an exhaustive compilation of all collections of these threatened animals, followed by biological surveys (i.e., actual field searches) for those species showing a high likelihood of being present. In 1976, the Indiana Chapter of the American Fisheries Society proposed to the Indiana Academy of Science that the Chapter compile these threatened species data. The AFS Chapter felt not only that the data were urgently needed, but also that the Chapter had within its ranks the expertise to do the job. The Academy agreed, and issued a grant of \$1400 for the production of a comprehensive document on the subject.

The Indiana Chapter of the AFS persuaded two of its members to take on this task. James Gammon analyzed the status of threatened fishes, and John O. Whitaker, Jr., took on the assignment of bringing together all the distributional data for mammals, birds, reptiles, and amphibians. The AFS Chapter would restrict its attention to these groups; no lower animals or plants were to be considered.

We assembled a working list of species to be considered from three primary sources. One of these was the 1972 Soil Conservation list of rare and endangered plants and animals. A second list (for amphibians and reptiles) was one prepared by the Society for the Study of Amphibians and Reptiles (SSAR) in 1972. The third source was a list of vertebrate animals received from the Department of Natural Resources in November, 1976. These three lists became our working roster, and species appearing on one or more of these three lists were reviewed in depth.

Most of this review entailed an intensive analysis of past distributional records of each species. In a few cases it appeared that the presumed rarity of the animal might be merely due to lack of spatial or temporal collecting effort. Further, previous collectors may have used less effective collection methods than those which were currently available. In these cases, actual field collections were made. (It should be noted, however, that field collection efforts were minimal.)

All previously published distributional data were compiled on the included species, and the results of any actual field collections were added to this data base. Other authorities on Indiana fauna were contacted, and their personal knowledge of unpublished occurrences of these animals was sought. Some were asked to review the first draft of the manuscript. They supplied a number of additions and corrections.

With these data assembled, Whitaker and Gammon made a critical judgment for each species. These judgments took one of three forms: 1) the status of the species (endangered or threatened) on the working list was most probably correct; 2) the species was moved from one category to another within the list (e.g., a species was listed as "Endangered" on the working list, but should

be lowered to "Threatened"); or 3) should be removed from the list entirely. On the basis of present distributional data, we would assign species on the *working list* to the categories indicated in Table I. Note, however, that we reluctantly abstained from a fourth category in these judgments, viz., *adding* species to the working list which were not originally on it.

The manuscript is now complete. It includes discussion of the distributional status for each species. We have included all records of which we are aware and we hope that we have eliminated much of the ambiguity concerning collecting localities of these animals. This does *not* mean, however, that the job is complete.

There should be a continuing review of the status of decimated and/or rare species. For example, Gammon and McReynolds wonder why such species as *Fundulus catenatus*, *Phoxinus erythrogaster*, and a few others were *not* on the list for which this review was made. Authorities such as the Indiana DNR and the Academy's Biological Survey Committee should constantly—or at least periodically—review the status of existing or potential candidates for the protected list. Only by a continuing monitoring of these animals will we be able to rescue those on the brink.

TABLE I *Base list of species of mammals, birds, reptiles, amphibians, and fishes of Indiana proposed for consideration for endangered or threatened status, and our recommended proposed status.*

Animals	Our Proposed Status
<i>Mammals</i>	
<i>Sorex longirostris</i> , Southeastern shrew	Omit from list
<i>Condylura cristata</i> , Star-nosed mole	Omit from list
<i>Myotis austroriparius</i> , Southeastern bat	Threatened
<i>Myotis grisescens</i> , Gray bat	Endangered
<i>Myotis sodalis</i> , Indiana bat	Endangered
<i>Plecotus rafinesquii</i> , Big-eared bat	Endangered
<i>Spermophilus franklinii</i> , Franklin's ground squirrel	Omit from list
<i>Geomys bursarius</i> , Pocket gopher	Omit from list
<i>Neotoma floridana</i> , Eastern wood rat	Threatened
<i>Reithrodontomys megalotis</i> , Western harvest mouse	Omit from list
<i>Sylvilagus aquaticus</i> , Swamp rabbit	Threatened
<i>Lynx rufus</i> , Bobcat	Endangered
<i>Taxidea taxus</i> , Badger	Threatened
<i>Birds</i>	
<i>Haliaeetus leucocephalus</i> , Bald eagle	Endangered

Animals	Our Proposed Status
<i>Falco peregrinus anatum</i> , Peregrine falcon	Endangered
<i>Dendroica kirtlandi</i> , Kirtland's warbler	Endangered
<i>Tympanuchas cupido</i> , Prairie chicken	Endangered (and possibly extirpated in last 5 years)
<i>Grus canadensis</i> , Greater sandhill crane	Threatened
<i>Pandion haliaetus</i> , American osprey	Threatened
<i>Reptiles</i>	
<i>Kinosternon subrubrum</i> , Eastern mud turtle	Endangered
<i>Clemmys guttata</i> , Spotted turtle	Threatened
<i>Pseudemys concinna</i> , Hieroglyphic turtle	Endangered
<i>Natrix erythrogaster</i> , Northern copperbelly	Threatened
<i>Thamnophis butleri</i> , Butler's garter snake	Endangered
<i>Opheodrys vernalis</i> , Western smooth green snake	Threatened
<i>Cemophora coccinea</i> , Scarlet snake	Threatened
<i>Tantilla coronata</i> , Crowned snake	Threatened
<i>Sistrurus catenatus</i> , Eastern massasauga	Threatened
<i>Amphibians</i>	
<i>Cryptobranchus alleganiensis</i> , Hellbender	Endangered
<i>Ambystoma platineum</i> , Silvery salamander	Threatened
<i>Ambystoma tremblayi</i> , Tremblay's salamander	Threatened
<i>Hemidactylium scutatum</i> , Four-toed salamander	Threatened
<i>Pseudotriton r. ruber</i> , Northern red salamander	Endangered
<i>Fishes</i>	
<i>Acipenser fulvescens</i> , Lake sturgeon	Endangered
<i>Coregonus artedii</i> , Cisco	Threatened
<i>Esox masquinongy ohioensis</i> , Ohio muskellunge	Endangered
<i>Esox m. masquinongy</i> , Great Lakes muskellunge	Endangered
<i>Clinostomus elongatus</i> , Redside dace	Endangered
<i>Notropis ardens</i> , Rosefin shiner	Threatened
<i>Notropis ariommus</i> , Popeye shiner	Endangered

Animals	Our Proposed Status
<i>Notropis fumeus</i> , Ribbon shiner	Omit from list
<i>Notropis shumardi</i> , Silverband shiner	Omit from list
<i>Cycleptus elongatus</i> , Blue sucker	Threatened
<i>Amblyopsis spelaea</i> , Northern cavefish	Endangered
<i>Chologaster agassizi</i> , Spring cavefish	Omit from list
<i>Typhlichthys subterraneus</i> , Southern cavefish	Endangered
<i>Ammocrypta asprella</i> , Crystal darter	Endangered
<i>Ammocrypta pellucida</i> , Eastern sand darter	Threatened
<i>Etheostoma camurum</i> , Bluebreast darter	Endangered
<i>Etheostoma swaini</i> , Swamp darter	Omit from list
<i>Etheostoma histrio</i> , Harlequin darter	Endangered
<i>Etheostoma maculatum</i> , Spotted darter	Endangered
<i>Etheostoma squamiceps</i> , Spottail darter	Endangered
<i>Etheostoma tippecanoe</i> , Tippecanoe darter	Endangered
<i>Etheostoma variatum</i> , Variegate darter	Endangered
<i>Percina evides</i> , Gilt darter	Endangered
<i>Percina uranidea</i> , Stargazing darter	Omit from list

Cottontail Rabbit Utilization of Artificial Burrows In East-Central Indiana

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Introduction

Changes in land-use associated with modern agriculture and expanding urbanization are destroying large areas of wildlife habitat including natural animal burrows or dens. When an animal's habitat is destroyed it is either driven from the area or it disappears altogether. As a part of habitat restoration for certain species of terrestrial mammalian wildlife in the midwest, an alternative to the destroyed dens must be provided. Man-made structures (i.e., artificial burrows) may be one alternative.

Time or seasons of year that an artificial burrow will be of most value to cottontails, *Sylvilagus floridanus*, is unknown. Vegetative cover type preferences of burrow-using cottontails have not been investigated. This preliminary investigation was conducted to determine if artificial burrows can be constructed that will be used by east-central Indiana cottontails.

Methods and Materials

The lay-up chamber of each artificial burrow was constructed from the upper portion of a 55-gallon steel drum. Each drum was cut transversely 41 cm below the top. On the top end of the drum a removable lid was attached and the open (bottom) end was secured to a square wooden frame. Each open wooden frame was constructed from 4" x 4" timbers to form a square foundation. A 10 cm diameter plastic soil drain pipe, 120 cm in length, was inserted into each of two opposing 13 cm diameter holes cut in the drums directly above the wooden frames. These pipes were used as entrance ways into the burrows and were arbitrarily aligned in a north-south direction as the burrows were constructed. The burrows were surrounded with fill-dirt up to the lids, covering the entrance tunnels to their openings. Ryegrass, *Lolium* sp., was sown on the fill-dirt.

The 16 burrows were placed at 50 m intervals in four adjacent vegetative cover types including fescue, *Festuca* sp., grassland; multiflora rose, *Rosa multiflora*, hedgerow; ungrazed deciduous forest; and woody wildlife plantings.

Captured cottontails were examined and ear tagged prior to release.

Results

A total of 56 inspections of each burrow was made during the winters of 1976-1977 and 1977-1978. Cottontail rabbits were the only wildlife species found in the burrows. There were 36 cottontail captures including seven individuals

captured two or more times. No cottontails tagged during the winter of 1976-1977 were recaptured the following winter.

Cottontails were captured in burrows in all study cover types. A Chi Square analysis was done to test the hypothesis that there was no difference in burrow utilization due to cover type. The hypothesis was rejected at the .01 significance level. A total of 75 percent of all captures was made in the forest and in the multiflora rose.

Results of regression analysis on capture versus mean air temperature gave an R value of -0.5. The presence of precipitation in some form may have more influence than air temperature on the incidence of burrow utilization by cottontails. Every cottontail capture occurred while rain or snow was falling or while snow covered the ground.

It appears that cottontails will utilize artificial burrows during times of active precipitation or snow cover. Frequency of usage of burrows constructed as described in this paper will vary with cover type.

Developmental Mortality of *Ambystoma tigrinum* (Amphibia: Urodela) in Northern Indiana

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Introduction

The only comprehensive study of developmental mortality in the eastern tiger salamander, *Ambystoma t. tigrinum* (Green) was by Anderson *et. al.* (1) in New Jersey. They determined that an average of 3.3% of the total number of eggs deposited survived to metamorphosis and that increased mortality occurred during gastrulation, hatching and metamorphosis. Mortality was greater in small than large masses. Low temperatures, predation and possibly pH significantly effected developmental mortality (1). The present paper provides the first data on developmental mortality in a midwest population of *A. t. tigrinum*.

Materials and Methods

The study pond, in northern St. Joseph County, Indiana, was described by Sever and Dineen (2). Briefly, the pond at maximum size is .25 ha and 60 cm deep, is filled by melting winter snows and rainfall, and usually holds water until July. It is in an open grassy field, but there are some trees around the border and considerable emergent vegetation in the shallow areas. During the spring of 1976, the pH was 6.8-7.0. *A. tigrinum* was the only salamander breeding in the pond, but the anurans *Pseudacris triseriata* and *Bufo americanus* were present. Sever and Dineen (2) estimated 1500-2000 *A. tigrinum* in the breeding population.

Prior to appearance of adult salamanders in February, 1977, 10 quadrats, each 2 m², were randomly established within the pond. Water depth was 26-33 cm in seven and 37-46 cm in three. Emergent vegetation was more abundant in the shallower quadrats, but all contained submerged branches and vegetation. After egg masses were deposited, the branch of vegetation to which they were attached was tagged and numbered. Eggs were studied from deposition to neurulation. The total number of dead eggs, living eggs, and the developmental stages of the living eggs were recorded every two days. Due to clarity of the water and large egg size, the eggs were easily examined with minimal handling.

Precipitation data were obtained from the U.S. Weather Bureau, South Bend, Indiana. Air, soil and water temperatures were recorded during the afternoon of each visit to the pond. A .01 probability level was used to determine statistical significance.

Results

The first eggs were noted on 11 March and the last new masses on 11 April. Between 11-16 March, 72% of the masses were deposited, and the remainder

between 29 March-11 April. These periods corresponded to times of high precipitation, mean ambient air temperatures greater than 10°C (except 4°C on 5 April) and water temperatures greater than 8°C (except 6°C and 5°C on 1 and 5 April, respectively). On any given day, there was no significant vertical temperature gradient in the pond.

A total of 861 eggs were present in 50 masses unevenly distributed among the 10 quadrats. The seven shallow water quadrats had 1-14 masses, the three deep ones 1-4 masses. The number of eggs per mass was 3-41 ($\bar{x}$ = 17.2, SD = 5.49). The variance in number of eggs per mass was highly significant among quadrats (F = 489.0).

Of the 50 egg masses, 34.6% disappeared before hatching. Significant increases in the number of clusters that disappeared were seen on 19 March and 5 April, days of low temperatures ($\bar{x}$ = 6°C on 19 March) following periods of optimal temperatures and intense egg-laying activity. These masses were assumed to have 100% mortality because most were in early gastrula prior to disappearing. Some mortality but less than 100% occurred in 59.4% of the masses and six percent of the clusters had no mortality to neurula.

One way analyses of variance among quadrats were highly significant for mean mortality between cleavage and gastrula (F = 450.2), gastrula and neurula (F = 308.2) and cumulative percent mortality from cleavage to neurula (F = 1605.4). Such results indicate that further testing is required to identify individual differences among quadrats. However, the quadrats were artificial units, and variance of mortality within the pond may be such that these quadrats are not the appropriate units for comparison.

Mean mortality between developmental stages based on grouped data from all quadrats is shown in Table I. The mean percent mortality was greater between cleavage and gastrula ($\bar{x}$ = 51.1%) than between gastrula and neurula ($\bar{x}$ = 36.0%). The mean cumulative percent mortality from cleavage to neurula was 74.3%.

TABLE I Mean percent mortalities and their standard deviations (SD) between developmental stages of *Ambystoma tigrinum*. Means are based on combined data on individual egg masses (N) from all quadrats.

Developmental stages	N	Mean %	SD
Cleavage-gastrula	50	51.1	29.6
Gastrula-neurula	37	36.0	18.3
Cleavage-neurula	50	74.3	16.4

Clutch sizes were arbitrarily grouped into nests of 1-10 eggs (18% of the total), 11-20 eggs (50%) and 21 eggs or more (32%). By inspection, there were no significant differences among these categories in cumulative percent mortality from cleavage to neurula.

The earliest deposited egg masses developed to hatching by 29 March. The remaining masses hatched between 11-16 April. Predation on egg masses was not observed.

Discussion

There is considerable variation in clutch size and mortality among egg masses throughout the pond. There are fewer clutches laid in deeper water, but variation in size and mortality of these clutches is similar to those laid in shallower water. The lower number of clutches in deeper water is probably the result of fewer suitable objects for clutch attachment.

The appearance of new egg masses throughout the period of this study supports Sever and Dineen's (2) findings that female *A. tigrinum* in this population oviposit asynchronously.

Many of the masses disappeared on 19 March and 5 April, days of lowered temperature following intense breeding activity. Anderson *et al.* (1) also reported that low temperatures increased developmental mortality in *A. tigrinum*.

Contrary to the findings of Anderson *et al.* (1), we found higher mortality between cleavage and gastrula than between gastrula and neurula. Also, we found no correlation between clutch size and mortality while Anderson *et al.* (1) reported higher mortality in smaller clutches. The majority of the masses observed by Anderson *et al.* (1) were larger than the masses observed in this study.

These differences may indicate distinct adaptations between Indiana and New Jersey populations resulting from different environmental influences. On the other hand, both our study and that of Anderson *et al.* (1) report on only one breeding season, and egg mortality at a given stage within a population may vary from year to year depending on environmental factors.

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Base Line Study of Lake Sno-Tip With Emphasis on Chemical Characteristics

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Abstract

Selected chemical and physical characteristics were routinely measured over the period of a year of Lake Sno-Tip, a five-acre lake located on the campus of Huntington College, Huntington, Indiana. Alkalinities, pH, Dissolved Oxygen, Nitrate-Nitrogen, and Total Phosphorus were among the chemical characteristics measured. A bathy-metric map and associated data were prepared, in addition to other physical measurements. The lake appears to exhibit stratification, high Total Phosphorus concentration, and other characteristics of an eutrophic lake.

Introduction

An important part of the early planning for Huntington College (called Central College, then) in 1896 was a lake 75 feet deep and one-half mile long. This lake was to be located along the north side of what is now known as Lake Street (3). In 1966, through the persistent efforts of two men, F. L. Tipmore and Gene Snowden, the lake became a reality. The lake, called Sno-Tip after its founders, falls considerably short of the original plans because it was built as a 1.98 hectare (4.89-acre) impoundment with a watershed of 155 acres.

The lake is surrounded by residential and forested areas. Water is supplied by five tiles, one of unknown origin and two creeks carrying run-off from forested, farming, commercial parking, and street areas. The lake bottom consists of clay and muck which had received considerable septic tank effluent for years prior to its construction.

Morphometry

A map of the lake showing sampling sites is presented in (Fig. 1). This map was prepared from an aerial photograph supplied by the U.S. Department of Agriculture. The depth contours were determined by taking soundings at specific locations on the lake. A range finder was used to determine the locations between two given points on opposite shores. Morphometric data will be found in Table I.

Methods

Temperature and Dissolved Oxygen measurements were made with an International Biophysics Corporation Field Model 490-051 meter. A homemade underwater photometer and a 20-cm. Secchi disk were used to measure light penetration. Nitrate-Nitrogen, pH, Alkalinity, and conductivity measurements were made in accordance with procedures found in "Standard

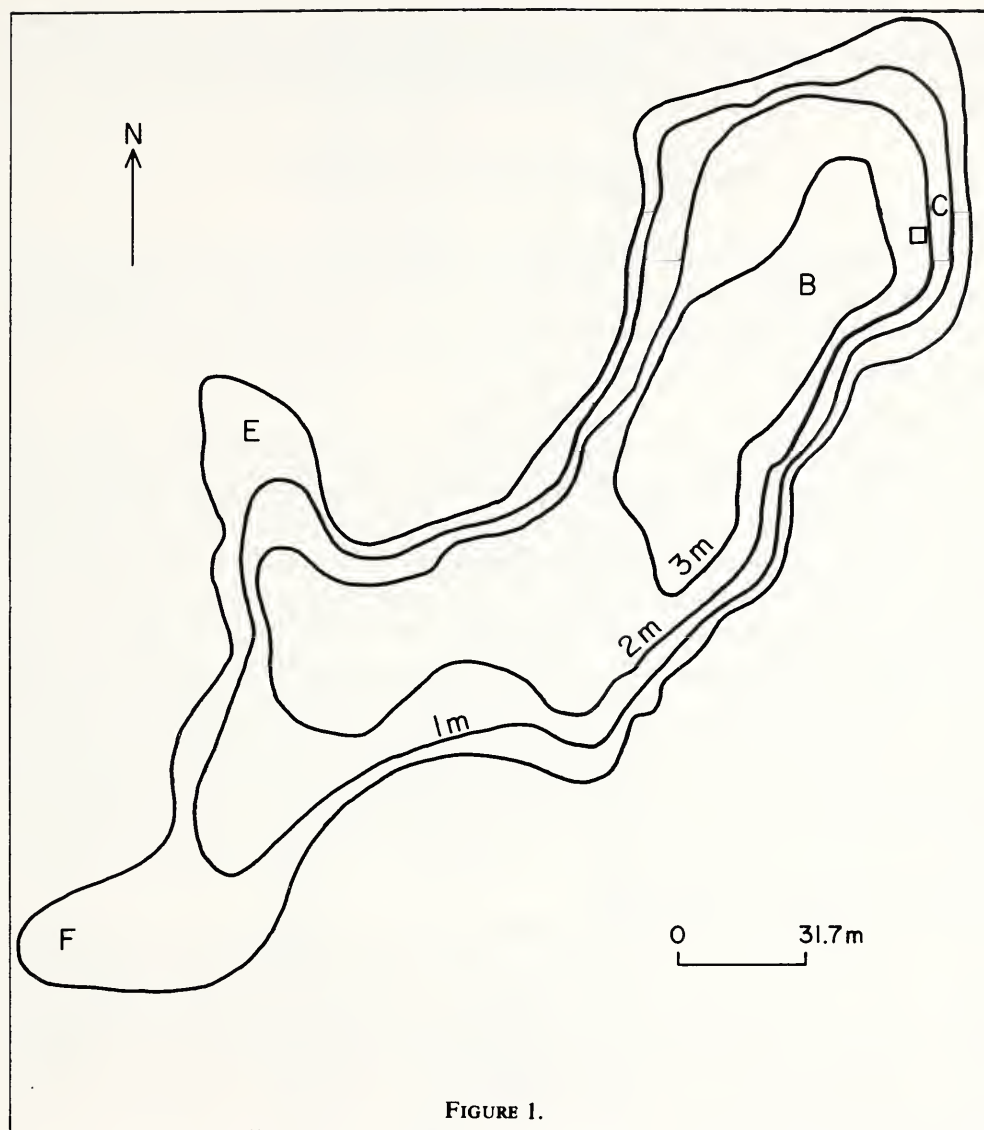


TABLE I *Morphometry of Lake Sno-Tip, September — July 1978*

Maximum Length	325 meters
Maximum Breadth	140 meters
Surface Area	4.89 acres
	1.98 hectares
Mean Width	61 meters
Length of Shoreline	840 meters
Shoreline Development Index	1.68
Maximum Depth	3.9 meters
Relative Depth	2.46%
Volume	28.85 acre-feet
	35,622 cubic feet
Mean Depth	1.8 meters
Volume Development	1.38
Percent Slope	4.91%
Watershed Area	155 acres
	62.75 hectares

Methods for the Examination of Water and Sewage" (5). Total Phosphorus was measured according to the method outlined in "Procedures for Wastewater Analysis" published by the Hach Chemical Company (2nd edition) (1).

Physical and Chemical Characteristics

Temperature

The temperature of the surface water followed a seasonal pattern for most of the year. However, the bottom water at 3.5 meters exhibited a difference of 8°C from surface water during the month of July 1978. Thermal stratification was evident between the spring (April) and fall (October) overturn.

Transparency

Secchi disk readings averaged 0.5 meters during September and October of 1977. During this period, tremendous amounts of phytoplankton were observed in the water. In June of 1978 after the addition of copper sulfate, Secchi depth reached a maximum of 2.75 meters. This indicates that light was reaching the bottom of most of the lake. Further, an underwater photometer revealed that on October 31, 1977, with a Secchi disk reading of 0.5 meters, one percent of the surface light reached a depth of one meter giving a factor of two times the Secchi depth. Using this factor of two, the Secchi depth of 2.75 meters observed in June would then yield a one-percent level at 5.5 meters. This depth is greater than the maximum depth of the lake; therefore, light in sufficient quantity for photosynthesis, did reach the entire bottom of the lake. This was confirmed by the pond weed (*Potamogeton* sp.) that appeared over the entire lake bottom.

Alkalinity, pH, and Conductivity

Alkalinity measurements along with pH and Conductivity will be found in Table II. The bottom water averaged lower in pH and higher in conductivity than did the surface water, thus indicating conditions of higher acidity and greater density. The high value of conductivity for the two sites E and F suggests that considerable dissolved ionic material and sediment is entering the lake through these inlets.

Dissolved Oxygen

Dissolved Oxygen measurements are shown in Table III for sampling site B. The ice was too thin in December to allow the Dissolved Oxygen to be determined. The data for that month, therefore, was taken from sampling site C (spillway). Ice covered the lake from early December through late March and reached a thickness of 46 cm. in early March. In every month except October (Fall overturn), bottom waters were much lower in Dissolved Oxygen than the first meter. These were definite signs of stagnation during stratification.

Nitrate-Nitrogen and Total Phosphate

Various phosphate parameters are usually defined by the method used for analysis (5). In this case the whole water sample was digested with persulfate followed by analysis for orthophosphate. The Nitrate-Nitrogen was measured by the cadmium reduction technique. Values beyond two decimal places were not considered significant due to limitations in the techniques and equipment used and were reported as less than 0.01 ppm concentration. Copper sulfate and Diquat were added during June and July of 1978. It is not known whether these

TABLE II *pH, Alkalinity, and Conductivity Measurements of Lake Sno-Tip from September to July 1977-78*

Sampling Site	Mean pH	Range	Conductivity 25°C u-mhos/cm	Range	Alkalinity (ppmCa CO ₃)	
					Mean Carbonate	Mean Bicarbonate
B Surface	8.4	9.2 (Oct) — 7.2 (Feb)	446	722 (Feb) — 277 (Sept)	14	126
B Bottom	7.8	9.2 (Oct) — 7.2 (April, Sept)	502	930 (Jan) — 298 (Oct)	12	172
C	8.5	9.2 (Oct) — 7.8 (Dec)	392	543 (May) — 272 (Sept)	15	116
E	8.5	9.8 (Dec) — 7.7 (Nov)	495	952 (Jan) — 272 (Sept)	25	151
F	8.5	9.6 (Dec) — 7.6 (Nov)	542	992 (Dec) — 283 (Sept)	30	174

TABLE III *Dissolved Oxygen (site B) of Lake Sno-Tip from September 1977 — July 1978*

	Sept	Oct	Nov	Dec	Jan	Feb	March	April	May	June	July
Surface	12.7 ppm	10.7	9.6	9.6*	7.1	1.4	0.7	15.0	12.5	8.7	6.1
0.5m											
1.0		10.5	9.7		4.0	1.0	0.5		12.5	9.6	6.2
1.5											
2.0		10.2			1.3	0.6	0.4		8.0	7.2	4.9
2.5				2.8*							
3.0		9.8	6.2		0.5	0.4	0.3			1.4	1.8
3.5	0.7							2.0	4.8		

*Taken from site C

TABLE IV *Total Phosphorus and Nitrate Nitrogen of Lake Sno-Tip from September 1977 — July 1978*

Sampling Site	Mean Total Phosphorus (ppm)	Range	Mean NO ₃ -N (ppm)	Range
B Surface	0.07	0.13 (Nov) — 0.03 (July)	0.08	0.64 (Jan) — <0.01 (June)
B Bottom	0.11	0.27 (Oct, Apr) — 0.05	0.15	0.64 (Jan) — <0.01 (June)
C	0.07	0.13 (Dec) — 0.01 (Oct)	0.13	0.60 (Dec) — <0.01 (June)
E	0.07	0.17 (Nov) — <0.01 (July)	0.23	0.74 (Jan) — <0.01 (June)
F	0.09	0.20 (Oct, Dec) — <0.01 (July)	0.12	0.66 (Dec) — <0.01 (June)

have an effect on the analytical method. The average Total Phosphate concentration does appear to be excessive when compared to other water systems (2). Sawyer reports that the phosphorus content should not exceed 0.015 ppm in "well behaved lakes" (4). The surface water of Lake Sno-Tip averaged 0.07 ppm with some values reaching as high as 0.20 ppm. Therefore, the lake can be expected to experience excessive algae and macrophyte growth. The stagnant bottom water is higher in phosphorus (0.11 ppm average) than the surface (0.07 ppm average). In addition, this water reached a value of 0.27 ppm just prior to the Spring overturn. Creek E contributes slightly less phosphorus (0.02 ppm) and approximately twice as much Nitrate-Nitrogen than does creek F. The watershed areas are somewhat different, creek E drains more agricultural and forested areas than does F. The watershed area of F is largely parking and residential. Significant amount of erosion occurs in both watershed areas.

Conclusions

From the results of this study, it can be stated that:

1. A stable condition of stratification exists between May and October.
2. The bottom water is rich in Total Phosphorus.
3. The contribution of Total Phosphorus is greater at creek F than E.
4. The Total Phosphorus concentration appears to be high enough (0.07 ppm average) to cause nuisance algae blooms and/or macrophyte problems.
5. The contribution of Nitrate-Nitrogen is greater at creek E than F.
6. Some effort needs to be made to control erosion in the watershed.

Acknowledgments

The author wishes to thank Dr. Thomas McComish of Ball State University for his interest, encouragement, and suggestions in connection with this study.

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Forage Legume Management Studies on Muck Soils¹

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Abstract

Most forage legumes do not persist for more than one year on muck soils in Northern Indiana. This lack of persistence is presumably due to the poorly drained conditions and low winter temperatures. Several experiments were conducted with Redland red clover (*Trifolium pratense* L.), Regal Ladino clover (*Trifolium repens* L.) and Empire birdsfoot trefoil (*Lotus corniculatus* L.) on the shallow Edwards muck soil located on the Pinney-Purdue Agricultural Center at Wanatah, Indiana. The experiments involved method of seeding, time of seeding, and fall mulching versus non-mulching.

The data obtained showed that some tillage is beneficial for legume establishment in a dense Kentucky bluegrass (*Poa pratensis* L.) sod even with the use of paraquat. Birdsfoot trefoil was successfully established from an August seeding although the common practice is to seed this species in the spring. Ladino clover did not survive the winter without the application of a straw mulch in October. Only a few red clover plants did not winter kill without mulch, however, birdsfoot trefoil plants appeared not only to survive, but started earlier and grew more vigorously with the protection of a mulch cover.

Introduction

Although the muck soils of northern Indiana are capable of producing high yields of corn, yields are frequently low due to adverse weather conditions. Often it is too wet in spring resulting in late planting of corn and the low-lying muck areas are subject to later summer frosts resulting in a shorter growing season. The low yields result in corn often being an uneconomical crop to grow on these muck soils of northern Indiana.

A possible alternative to grain production is to seed these muck soils to forage crops to be used as pasture or hay. Many cool-season forage grasses are known to be well adapted to muck soils. This is especially true of Kentucky bluegrass (*Poa pratensis* L.). This species soon predominates on these muck soils if taken out of grain production. The problem is to find a forage legume which will persist on the acid, poorly drained muck soils and also be able to compete with Kentucky bluegrass.

The following study was initiated in the spring of 1977 to determine the effect of seedling management on the establishment and persistence of birdsfoot trefoil, red clover, and ladino clover. These three legumes, and especially

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birdsfoot trefoil (1), were known to be the forage legumes most likely to succeed on these shallow muck soils.

Materials and Methods

Three experiments were initiated on the Pinney-Purdue Agricultural Center at Wanatah, Indiana in 1977. The soil type was an Edwards muck.

Experiment 1. Empire birdsfoot trefoil (*Lotus corniculatus* L.) was broadcast seeded on May 1, 1977 into an established stand of Kentucky bluegrass at the rate of 11.2 kg/ha. A 2 x 3 factorial set of treatments was imposed consisting of two soil fertility levels and three methods of handling grass competition. Fertility levels were unfertilized and 336 kg P₂O₅ and 336 kg K₂O/ha spring applied. Grass competition treatments were; check or no control attempted, paraquat applied 10 days prior to seeding legume at rate of 1 l/ha, and clipping at 1-2 cm above soil surface just prior to legume seeding. The plots were 2 by 3 m and replicated 4 times.

Experiment 2. On May 1, Redland red clover at 16.8 kg/ha and Regal ladino clover at 2.2 kg/ha were seeded into an established Kentucky bluegrass sod. Plots 3.3 by 5 m were established and paraquat at the rate of 1 l/ha was applied to half of the plots on April 20. The experimental area was rototilled just prior to seeding. Three replications were employed.

Experiment 3. This experiment was initiated to determine the feasibility of an August seeding of Empire birdsfoot trefoil. Prior to seeding on August 18, an area 5 by 7 m was rototilled, divided into 4 replications, and broadcast seeded by hand and covered shallowly with a rake.

On October 20, half of each plot of the August seeded birdsfoot trefoil was mulched with wheat straw and anchored in place by criss-crossing the mulched areas with twine. The red clover and ladino clover plots were divided in thirds, 1/3 was clipped and straw mulched, 1/3 was clipped and clippings removed, and 1/3 was not clipped or mulched. The mulch remained in place over winter and was removed on April 6, 1978.

Stand counts in the three experiments were obtained by randomly throwing a 20 by 50 cm quadrat.

Results and Discussion

Experiment 1. Stand counts of the birdsfoot trefoil nine weeks after seeding revealed that close clipping or the application of paraquat to bluegrass was necessary for establishing birdsfoot trefoil in a bluegrass sod (Table I). No plants survived in the plots that were not clipped or sprayed with paraquat. Spraying with paraquat resulted in nearly three times the population of trefoil seedlings as compared to close clipping of the bluegrass.

Visual estimates revealed that eighteen months after seeding birdsfoot trefoil in the paraquat treated plots, birdsfoot trefoil comprised slightly over 50% of the mixture. The close clipping resulted in a mixture of 28% birdsfoot trefoil and 72% Kentucky bluegrass.

TABLE I *Effect of seedling management on the establishment of Empire birdsfoot trefoil sod-seeded in a Kentucky bluegrass sod on Edwards muck soil at Wanatah, Indiana, 1977-78.*

Stand Evaluation	Check	Clipped	Paraquat
Plants/ Plot*	0	8.8	22.4
% Birdsfoot trefoil**	0.1	28.1	54.0

*Ave. of 4 reps 9 weeks after seeding.

**Ave. of 4 reps 18 months after seeding.

Experiment 2. As shown in Table II, the beneficial effect of paraquat when sod-seeding red clover and ladino clover was very similar to that obtained in Experiment 1 with birdsfoot trefoil. Nine weeks after seeding red clover and ladino clover, the number of plants in the plots sprayed with paraquat was approximately three times that of the plots receiving no paraquat (Table II). However, by late summer the foliar growth of the plots which did not receive paraquat appeared to be very similar to those sprayed with paraquat (visual appraisal).

TABLE II *Effect of treating Kentucky bluegrass sod with paraquat at time of seeding on the establishment of sod-seeded red clover and ladino clover on Edwards muck soil at Wanatah, Indiana, 1977.*

Species	-Paraquat	+ Paraquat
	Plants/0.1m ²	
Red clover	4.3	13.4
Ladino clover	1.1	3.3

Ave. of 3 reps 9 weeks after seeding.

Experiment 3. August seeding of Empire birdsfoot trefoil was successful despite an unusually cold winter in 1977-78 (Table III). The late fall application of a straw mulch did not benefit the trefoil seedlings. It appeared that the soil in the unmulched plots warmed up quicker and enabled the seedlings to start growing earlier and more vigorously in the spring than the plants that had been mulched.

TABLE III *Effect of a fall applied straw mulch on winter survival and vigor of Empire birdsfoot trefoil seedlings grown on Edwards muck soil at Wanatah, Indiana, 1977-78.*

Treatment	Crowns*	Stems	Flowers	Pods
	Per 0.1m ²			
Mulched	2.3	44.0	0	8.5
Unmulched	4.3	498	3.3	74

*Ave. of 4 reps 1 year after seeding.

The effect of mulching red clover and ladino clover was very striking. Ladino clover was completely winter-killed without a late fall applied mulch. A few red clover plants survived without mulch but not enough to warrant

keeping the stand. However, an excellent stand of both species remained in the plots which were covered with straw mulch.

These data suggest that possibly red clover and ladino clover would overwinter if grown in association with a grass which would provide a mulch to protect the clover plants. Also, grazing in the fall may be detrimental to winter survival of red clover and ladino clover. However, these results imply that heavy fall grazing of a trefoil-bluegrass pasture may be beneficial since the removal of residue by grazing would permit earlier spring growth of the trefoil, which is very winter-hardy.

Conclusions

The following conclusions are based on the data reported in this paper.

1. When sod-seeding a legume in a cool-season grass sod such as Kentucky bluegrass, it is necessary to reduce the competition of the grass by means of one, or more, of the following practices:

- a. Some type of tillage for partial destruction of the sod.
- b. Application of paraquat.
- c. Close clipping or heavy grazing prior to seeding.

2. A late summer seeding of birdsfoot trefoil can be successful if properly managed.

3. A fall applied straw mulch will reduce winter-killing of red clover and ladino clover.

4. Birdsfoot trefoil seedlings are very winter-hardy and mulching is not necessary. In fact, heavy fall grazing may be beneficial since the removal of residue permits earlier spring growth.

5. Birdsfoot trefoil appears to be the only forage legume adapted to muck soil in northern Indiana.

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Edaphic Relationships of Spicebush *Lindera benzoin* (L.) Blume

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Introduction

The research was performed in Ginn Woods, a 67.5 h beech-maple climax community located approximately ten miles north of Muncie and 0.5 mile southwest of the Missisniewa River. The stand is in Section 18, T22 N, Rio E in Union Twp. of Delaware County, Indiana. The predominant soil types in the woods are a Blount silt loam and a Pewamo silty clay loam, both developed from Late, Late Wisconsin glacial till (3). The woods is presently owned by Ball State University and is primarily utilized for field research by the Department of Biology. Since the purchase of the woods in 1972 there has been no disturbance by cutting, grazing or fire. The only logging since the mid-1800's was the removal of 16 mature black walnuts in 1967.

In east central Indiana, spicebush, *Lindera benzoin* is most commonly found in the highest densities on upland moist depressional sites in Mixed Mesophytic and Beech-Maple Forest types. These sites are poorly drained but not normally ponded for extended periods of time. The spicebush occurs in essentially pure stands within the shrub stratum.

Methods

Three spicebush study sites were selected within Ginn Woods for evaluation. The selection was based on the amount of soil surface area covered by the shrubs related to the density of same. The three subcommunities with the highest coverage and density were used. The three communities averaged 20 plants per site and an average area of 136 m² per site. East-west transects were oriented through each of the study areas and stations established on the transects at one m intervals. The transects extended through fingers of Pewamo silty clay loam and onto adjacent higher areas of Blount silt loam. Bouyoucos moisture blocks and Y.S.I. temperature thermistors were buried at each station at 5 and 20 cm depths to monitor available soil moisture and soil temperature daily through the growing season of 1977. Air temperature at 25 cm above the soil surface was also recorded daily for each station. Soil samples were removed at each point for lab analysis of texture and nutrient levels.

Also at each station samples of spicebush plant tissues were made at various phenological stages. The stages of development and organs sampled were as follows: dormancy (shoot), mature bud (bud and shoot), anthesis (flower, shoot and immature leaf) and at leaf abscission (shoot and freshly fallen leaf).

Soil cores were oven dried at 50°C for 24 hours and ground with a Waring blender prior to nutrient analysis. Plant tissue samples were dried at 65°C for two hours and ground with a Wiley mill with a 20 mesh screen. Nitrate nitrogen for both plant tissues and soils was determined by the modified Kjeldahl method

(2). Total phosphorous in plant tissues was analyzed by the Molybdenum Blue Method (2). Soil phosphorus values were obtained using the Sodium Bicarbonate Method of Olson (2). Eight trace minerals, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn were analyzed for both soil and plant organ samples following the Acid Digestion Procedure of Perkin-Elmer (4). Processed samples were filtered and diluted with de-ionized water for determinations in ppm with Atomic Absorption Spectrophotometer. Textural analysis of the soil samples followed the standard hydrometric methods of Bouyoucos.

Results

Tree density and canopy cover (1) were similar at all three study sites therefore insignificant differences prevailed between sites for available soil moisture. Spicebush density was also similar with 22 plants at the 144 m² Site I, 19 for 132 m² at Site II and 19 for 132 m² at Site III. Fluctuations in moisture were correlated with precipitation; the greatest differences at the 5 cm depth. A direct relationship between available moisture and station location was also evident with the most depressional and higher clay content stations with the highest available soil moisture levels. Soil temperature corresponded as anticipated with air temperature. Lower growing season soil temperatures occurred at the 20 cm depth and at the more depressional stations on the Pewamo soil type.

Consideration of the data on trace mineral levels in the soil at each station or in plant organ tissues at the phenological stages indicated no correlation. Wide variations occurred in mineral levels between stations on the same soil types and at the same time period. The two macronutrient soil analyses however gave several positive correlations. All three sites were remarkably similar in these correlations. Nitrate levels varied from an average of 0.20% by weight on the Blount to 0.27 on the Pewamo. Total phosphate, though at lower levels, ranged from an average of 0.09% on the Blount to 0.16 on the Pewamo. Tissue sample analyses for the two macronutrients gave the highest nitrate values, a mean of 0.31%, for shoot samples and the lowest, 0.23% for leaves. These values remained relatively unchanged through the growing season. Phosphate levels were inverted in that the highest levels, 0.16%, were in the leaves and the lowest, 0.09%, in the shoots.

The highest spicebush densities did occur on the Pewamo silty clay loams. The higher densities appeared to be related to available soil moisture through the growing season due to drainage and higher clay contents. Soil phosphate levels also seemed to have a positive effect on the distribution of spicebush in Ginn Woods.

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ENTOMOLOGY

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Abstracts

Polyacrylamide Gel Electrophoresis of Esterase Enzymes in the Insect Order Collembola. JOHN W. HART and BETTY D. ALLAMONG, Departments of Natural Resources and Biology, Ball State University, Muncie, Indiana 47306.——Polyacrylamide gel electrophoresis was used to study B- and C-esterases in five species of Collembola. E_f values were computed for zymograms of 105 individuals and compared both within and between species. Certain bands, three to six in number, were found to predominate in zymograms of individuals of each species. Different bands predominated among the five species studied, and it was concluded that enzyme differences between species would be valuable in concert with classical morphology in the determination of species in this order.

The Genus *Desmopachria*, a Taxonomic Nightmare (Coleoptera: Dytiscidae). FRANK N. YOUNG, Biology Department, Indiana University, Bloomington, Indiana 47401.——Most of the small water beetles of the genus *Desmopachria* are adapted for burrowing in the organic debris at the margins of freshwater ponds, lakes, and slow streams. A few tropical species live in water holding plants such as the bromeliads. Many species resemble tiny plant seeds more than insects. Color, size, and general shape are the only external characters for classification, and even scanning electron microscopy does not reveal any integumental features of value. The external male genitalia, however, prove to be both unique in structure and very diverse in different species. On the basis of the genitalia, four species are recognizable in Florida, three others in Mexico and Texas, and three in Brazil, all of which are nearly indistinguishable on external examination. These species are truly pseudosiblings of which the males cannot even be distinguished from females by external characters. A few species, in the western U.S. and Mexico have developed color patterns on the dorsum which are considered disruptive coloration, some tropical species have the elytra spotted or fasciate, but the majority are remarkably similar externally. All must be dissected before a positive identification can be made.

Mosquito Studies in Delaware County, Indiana, 1978. I Larval Studies. ROBERT M. KRUGER, Department of Biology, Ball State University, Muncie, Indiana 47306 and ROBERT R. PINGER, Department of Physiology and Health Science, Ball State University, Muncie, Indiana 47306.——A qualitative study of mosquito species in Delaware County was made based on larval collections. The study was designed to update the 1964 mosquito larval study by Dr. R. E. Siverly

and to lay the foundation for further mosquito research such as arbovirus and dog heartworm studies.

Larvae were collected from April through September, 1978, using a white enamel dipper, and transported to the laboratory for species identification. Samples were reared to adulthood, when necessary, to confirm larval identification. Specimens from selected sites were preserved and cataloged for future reference.

A total of 3000 individual larvae were identified from 54 different sites. Of the 19 species identified, 4 represent new county records, *Culex tarsalis*, *Orthopodomyia alba*, *Aedes hendersoni*, and *Psorophora howardii*. *Culex pipens* and *Aedes vexans* were the most frequently encountered.

Mosquito Studies in Delaware County, Indiana 1978. II Light Trap Studies. ALICE K. THOMAS and ROBERT R. PINGER, Department of Physiology and Health Science, Ball State University, Muncie, Indiana 47306._____Four New Jersey light traps were operated daily from April to October to sample adult mosquito populations in Delaware County, Indiana in 1978. Supplementary adult collections were made from time to time with Dry-ice baited CDC miniature light traps.

Approximately 10,000 mosquitoes comprising 22 species were collected and identified. The first mosquito collected, *Culiseta inornata*, was captured on April 9th. *Aedes vexans*, *Aedes trivittatus* and *Culex pipiens* predominated in the New Jersey light trap collections. Peak adult populations occurred during the second half of July.

Culex tarsalis was collected at three different sites in Delaware County and was also taken in a CDC trap in neighboring Grant County. These represent new county records for this important disease vector. *Aedes dorsalis*, reported from a single site in Delaware County in 1972 was collected at several new sites in 1978.

The Susceptibility of Indiana Mosquito Populations to Five Insecticides Commonly Used in Control Programs. WILLIAM E. BLUE, DIANE BERGOCH, and MICHAEL J. SINNSKO*, Indiana State Board of Health, Indiana._____Several counties in the State of Indiana have started mosquito control programs as a result of the epidemic of St. Louis Encephalitis in 1975. Since mosquito populations in some of these areas have never been subjected to prior pesticide selection pressure, this has provided an opportunity to collect susceptibility base-line data. *Culex pipiens* and *Aedes vexans* populations have been tested for susceptibility to malathion, temephos, fenthion, propoxur and Flit-MLR^R in widely scattered areas of the State. All mosquitoes were found to be highly susceptible to all insecticides. These data will be used as a base-line for regular testing in the future.

Introduction of *Rhinocyllus concicus* (Froelich) (Coleoptera; Curculionidae) for Biocontrol of Musk Thistle in Indiana. JOHN J. FAVINGER, Indiana

*Will present paper at Fall meeting.

^RTrade Name.

Department of Natural Resources, Indianapolis, Indiana 46204._____The musk or nodding thistle (*Carduus nutans* L.) is an introduced biennial weed that has become established along roadsides and in pastures and waste areas in many southern Indiana counties. Appreciable control has been effected in several states, notably Montana, Virginia and West Virginia, by the introduction of a seed-feeding weevil *Rhinocyllus conicus* (Froelich) host specific to four genera of thistles, *Carduus*, *Cirsium*, *Silybum* and *Onopordum*. In July 1977 approximately 600 adult weevils from Pulaski County Virginia were released by Division of Entomology personnel at dense thistle sites in Switzerland, Jefferson and Johnson Counties. An additional 1000 adult weevils were obtained from the same source and released at one prime site in Switzerland County May 22, 1978 as well as in Johnson County. Adult weevils were recovered from infested heads later in the summer at both major release sites. No recoveries have been made to data that can be attributed to the 1977 releases. Additional summer adults obtained from Monroe County, West Virginia were released July 25 and 29 at three Ohio County sites and in Johnson County.

Insect Control on Vegetables by Companionate Planting. ALAN C. YORK and KATHY O'GUIN, Purdue University, West Lafayette, Indiana 47907._____In garden size plots in which vegetable plants were surrounded with companionate plants, or sprayed with home remedy sprays or carbaryl, the following differences were observed. On cabbage, *Pieris rapae* was significantly reduced in number using a garlic mixture spray versus the companionate plants, onions, tarragon, dwarf marigold, nasturtium, or untreated. None of the treatments had any significant effect on *Trichoplusia ni*. On cucumbers, no significant differences existed between treatments with respect to cucumber beetles or yield. On snap beans, least damage occurred in the carbaryl treatment, but yields were not significantly different. Significantly fewer *Epilachna varivestis* larvae occurred in the garlic/hot pepper spray treatment than with dwarf marigold or summer savory. No differences existed in the number of *E. varivestis* egg masses. Differences in *Empoasca fabae* numbers were erratic between treatments. On eggplant fewest flea beetles and feeding scars occurred in the carbaryl treatment. Other treatments did not differ from one another including the untreated.

ULD applications of synergized pyrethrins for stored product pests. KAREN L. MCINTOSH, Purdue University, West Lafayette, Indiana 47907._____ULD applications of synergized pyrethrins were evaluated for control of rice weevils and confused flour beetles given various levels of protection. The distribution of droplets were also studied to examine the relationship between insect mortality and droplet penetration into harborage areas.

Ecomorphosis in *Proisotoma vesiculata* Folsom

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Introduction

A number of species in the Order Collembola, particularly in the family Isotomidae, exhibit a phenomenon known as ecomorphosis. During late spring, apparently in response to environmental factors such as rising temperatures and lowered soil moisture, individuals undergo morphological and physiological changes. The most spectacular change in many species is the appearance of four to six stout spines (usually papillate) dorsally on the rear of the abdomen. Stach (1966) erected a genus, *Spinisotoma*, to accommodate Isotomidae bearing such spines. For a review of ecomorphosis in Collembola, including a list of species assigned to the genus *Spinisotoma*, see Cassagnau (1956, 1971), Cassagnau and Fabres (1968), and Wonio and Pedigo (1974).

Folsom (1937) described *Proisotoma vesiculata* and commented in his description, "At the extremity of the dens is a large lateral transparent bladderlike dilation extending beyond the mucro."

This study reports an ecomorphic form of *Proisotoma vesiculata* and presents life history data which suggests that the vesicle (Fig. 1C) observed by Folsom is only present during a very short part of the life cycle (possibly during only one instar).

Results and Discussion

During 1974 and 1975 four individuals of a species of *Proisotoma* subgenus *Appendisotoma* were collected. They were considered to be ecomorphic because of six distinctive papillate spines borne dorsally on the last abdominal segment (Fig. 1A). The specimens closely resembled *Proisotoma (Appendisotoma) vesiculata*, a species not known to occur in Indiana, but differed in possessing abdominal spines and lacking dental vesicles (Fig. 1B).

In 1976, collections were made on 13 occasions—June through December. From June until late October only ecomorphic forms were taken, but on October 24 two slides were made in which ecomorphic individuals were in the process of molting from the ecomorphic form (with six large abdominal spines and no vesicle) to the normal form of *Proisotoma vesiculata* (without spines and with a vesicle).

During the period of study, 1974-1978, 56 collections were made and 665 slides were examined. Table I summarizes life cycle data. First instar young could not be identified with certainty, but considering that spermatheca were observed in the genital tract of males March 13, and first instar *Proisotoma* were present in the collections about April 1, it was reasonable to conclude that eggs were deposited about the last week in March. The molt from normal young to ecomorphic young took place for the most part in May and early June. By the

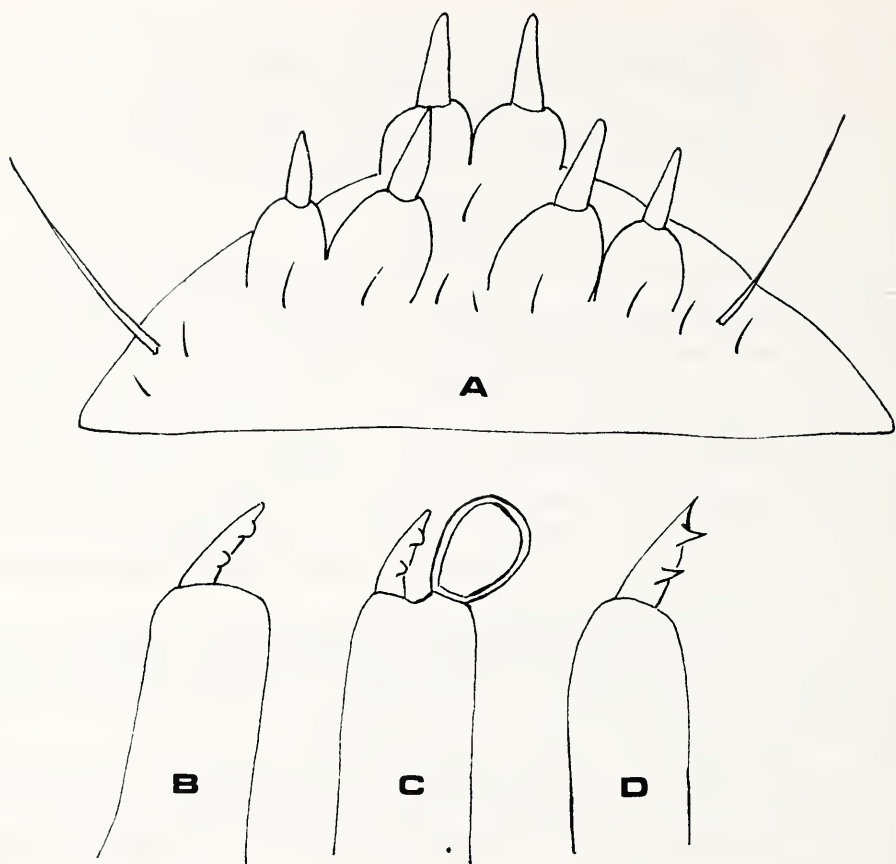


FIGURE 1. *Proisotoma vesiculata*. (A) Dorsal abdominal spines of an ecomorph. (B) Tip of dens and mucro of an ecomorph. (C) Tip of dens showing vesicle and mucro. (D) Tips of dens and mucro of adult.

first of July only ecomorphic forms were taken. Starting in late October, the ecomorphic forms molted to normal forms, and at this stage the vesicle first appeared. No hypothesis was formed as to its function, but it was only observed during October, November, and December. All mature adults collected in March were without the vesicle.

TABLE I Seasonal distribution of normal and ecomorphic young and adult *Proisotoma vesiculata*.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Eggs												
Young (normal, w/o vesicle)												
Young (normal, w/ vesicle)												
Young ecomorphic)												
Adults (w/ vesicle) ¹												
Adults (w/o vesicle)												

¹Type specimens were collected December 7, 8.

In a separate study (Gary Bannon, unpublished) the electron microscope was used to investigate the ultrastructure of the digestive tracts of normal and ecomorphic individuals of *Proisotoma vesiculata*. Bannon's conclusions agreed

with those of Cassagnau and Fabres (1968) who found that the mesenteron of ecomorphic forms was highly abnormal. It is probable that ecomorphic forms do not feed but depend on fatty deposits in the body as sources of energy, and in sections of ecomorphs examined by Bannon, numerous fat bodies were present. If ecomorphs do not eat and hence do not grow, they probably do not molt during ecomorphosis, and indeed, no instance was found in this study of an ecomorph to ecomorph molt.

It is difficult to determine the arrival of sexual maturity in Collembola, for the presence of a genital opening may be noted a number of weeks in advance of egg laying or sperm deposition. It should be noted also that sexually mature individuals continue to molt (Christiansen, 1964). With the exception of three large ecomorphic individuals which showed evidence of genital openings (one female, two males), all ecomorphs were juveniles. Even after the molt from ecomorphic to normal form, most individuals remained in the juvenile stage. It is unfortunate that severe winter weather in Indiana during 1976 and 1977 precluded sampling during January and February, for it is during this period that the vesicle is lost and sexual maturity arrives.

This study is the first to report ecomorphosis in the genus *Proisotoma*, but two additional undescribed species of *Proisotoma* (*Appendisotoma*) which exhibit this phenomenon are under study, and it is possible (if not probable) that all of the members of this subgenus exhibit ecomorphosis.

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Insects and Other Arthropods of Economic Importance In Indiana During 1978¹

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Introduction

A heavy snowfall at the end of November in 1977 was the start of a long, hard winter which included the coldest February in the recorded meteorological history of the state. While snow cover favors most insects in this area, the long winter, followed by a wet spring, was not so kind to overwintering crops. Winter wheat, with acreage already reduced for other reasons to 900,000 acres, suffered winter damage to the extent that about 17% of even that small acreage was not harvested for grain. Alfalfa also suffered; two fields regularly surveyed lost more than half their stand due to the winter (and unwise cultural practices).

The large amount of volunteer corn in field corn and especially in soybean fields was reportedly weather-related: the kernels in the field that would ordinarily have sprouted and died or rotted during the freeze-thaw cycles were preserved intact to sprout at the proper time. Late planting of corn due to wet weather tended to favor weed control as well as the European corn borer and to inhibit corn rootworm numbers. The warm, and sometimes very warm summer with often spotty but usually adequate moisture speeded development of most crops, and nearly ideal harvest conditions in the fall combined to produce corn yields better than anticipated earlier in the year.

Corn and Small Grains

The western corn rootworm (*Diabrotica virgifera* LeConte) was not the problem in corn that it was last year. The first adult appeared later, (Table II gives available developmental data on this and other insects), populations peaked later (by more than a month as indicated by sticky trap catches) and at lower levels than in 1977. Beetles/trap/day from mid-July to 31 Aug. were 2.0, 3.2, 2.8, 15, 12.9 and 12.7 (weekly means) in an untreated Tippecanoe Co. corn field. From counts of adults made from 7-18 Aug., the following figures are means/stalk/district of both the northern (*D. longicornis* (Say)) and western corn rootworm adults: (The percent that were westerns is given in parentheses) NW—0.78 (71); NC—0.72 (97); NE—0.71 (95); WC—0.51 (52); C—0.84 (36); and EC—0.81 (48).

Economic damage to silks by adults was probably rare; and because climatic conditions for the corn were favorable, economic losses due to larval feeding, even when relatively severe, were difficult to demonstrate. (Table I records the new counties which this and other insects invaded during the year.)

The European corn borer (*Ostrinia nubilalis* (Hübner)), however, reached larval numbers this year nearly double the highest recorded since the 1961

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TABLE I *New County Records for 1978*

Insect	New County Distribution
<i>Bathyplectes anurus</i> (Thomson)	Morgan
<i>Bathyplectes curculionis</i> (Thomson)	Pulaski, Whitley, Allen, Huntington, Wells, Adams, Fountain
<i>Diabrotica virgifera</i> LeConte	Orange, Washington, Bartholomew, Jefferson, Jennings
<i>Diaparsis</i> , sp. n.	Shelby, Union, Lawrence, Jefferson
<i>Lemophagus curtus</i> Townes	Huntington
<i>Microctonus aethiopoides</i> Loan	Warren, Jay
<i>Tetrastichus julis</i> (Walker)	Marshall, Decatur, Randolph, Union, Rush, Fayette, Dubois, Perry, Crawford, Floyd, Clark, Jefferson, Ohio, Scott, Switzerland

TABLE II *Date of 1st appearance and/or peak population of some insects*

Insect	Stage	First	Peak(s)	County	Data Source
Apple maggot	Adult	27 Jun		Tippecanoe	NJ mosq. tr.
	Adult		20-26 Jul	Knox	Bait trap
Armyworm	Adult	8 Apr		Lawrence	BL trap
	Ad. 1st flight		18-24 May	Tippecanoe	BL trap
	Ad. 2nd flight		6-12 Jul	Tippecanoe	BL trap
	Ad. 3rd flight		24-30 Aug	Tippecanoe	BL trap
Black cutworm	Adult	13 Apr	6-12 Jul	Lawrence	BL trap
<i>Chrysopa carnea</i>	Adult	7 Apr		Tippecanoe	NJ mosq. tr.
<i>C. oculata</i>	Adult	30 May		Tippecanoe	NJ mosq. tr.
<i>C. rufilabris</i>	Adult	31 May		Tippecanoe	NJ mosq. tr.
<i>C. nigricornis</i>	Adult	1 Jun		Tippecanoe	NJ mosq. tr.
<i>Chrysops callidus</i>	Adult	31 May		Vermillion	Bait
<i>C. cincticornis</i>	Adult	27 May		Tippecanoe	Bait
<i>C. niger</i>	Adult	31 May		Vermillion	Bait
Codling moth	Ad. 1st flight	11-17 May	18-24 May	Knox	Pheromone
	Ad. 2nd flight		23-28 Jun	Knox	Pheromone
	Ad. 3rd flight		24-30 Aug	Knox	Pheromone
Corn earworm	Adult	17 Jun		Lawrence	BL trap
European corn borer	Adult	24 May		Knox	BL trap
	Ad. 1st flight		1-7 Jun	Tippecanoe	BL trap
	Ad. 2nd flight		10-16 Aug	Tippecanoe	BL trap
	Ad. 3rd flight		7-13 Sep	Tippecanoe	BL trap
Meadow spittlebug	1st instar	11 Apr		Harrison	Observed
Mexican bean beetle	Adult	17 May		Jackson	Observed
	Egg	7 Jun		Lawrence	Trap crop
N. corn rootworm	Adult	2-12 Jul	10-17 Aug	Tippecanoe	Sticky tr.
Obliquebanded leafroller	Ad. 1st flight		18-24 May	Knox	Pheromone
	Ad. 2nd flight		1-6 Sep	Knox	Pheromone
Spotted lady beetle	Ad. 1st flight		20-26 Jul	Tippecanoe	Sticky tr.
	Ad. 2nd flight		10-17 Aug	Tippecanoe	Sticky tr.
	Ad. 3rd flight		1-6 Sep	Tippecanoe	Sticky tr.
Variegated cutworm	Egg	25 Apr		Harrison	Ovip. trap
	Adult	5 May		Lawrence	BL trap
	Ad. 1st flight		1-12 Jul	Tippecanoe	BL trap
	Ad. 2nd flight		24-30 Aug	Tippecanoe	BL trap
W. corn rootworm	Adult	6 Jul	7-14 Sep	Tippecanoe	Sticky tr.
Yellowstriped army	Adult	4 Jun		Tippecanoe	BL trap
Zimmerman pine moth	Adult	17 Aug		Tippecanoe	Research

survey. The fall (overwintering) 1977 larval population was high (68/100 stalks). Apparently there was sufficient corn of sufficient maturity for the resultant females, although heavy damage was occasionally observed in some of the early planted fields as a result of the spring oviposition. And when this generation was ready to oviposit, there was much late-planted corn available. The summer flight was heavy, and large numbers were collected in light traps from the end of July to mid-September.

In the fall survey two counties, Pulaski and Marshall, averaged more than 6 larvae/stalk, nearly the entire northern half of the state averaged higher numbers than at any time in survey history, and the state averaged 199.6 larvae/100 stalks. Larvae were also collected from jimsonweed (*Datura stramonium* L.) and velvetleaf (*Abutilon theophrasti* Medic.), and in a field of oats in Randolph Co. about 5% of the stems were infested.

A few corn fields, in SW Indiana primarily, were damaged by the black cutworm (*Agrotis ipsilon* (Hufnagel)) although poor germination due to other causes was sometimes blamed on this species. And the northern border of the state formed the southern edge of an armyworm (*Pseudaletia unipuncta* (Haworth)) invasion of larger proportions more seriously affecting Michigan. Attacks by earworms were also minor; this year the corn earworm (*Heliothis zea* (Boddie)) was the more common in ears, whereas in 1977 the fall armyworm (*Spodoptera frugiperda* (J. E. Smith)) predominated.

Mention should also be made of a carabid beetle, *Geopinus incrassatus* Dejean, adults of which were associated with small areas in a field of corn where no corn came up. This burrowing has not been implicated in damage to crops before, and may not have been this time, but indications were rather strong.

Late-planted corn tends to harbor large corn leaf aphid (*Rhopalosiphum maidis* (Fitch)) populations, especially when contrasted with an early-planted year like the last. The totals this year were not greatly different from those of last year—32% infested in 1977 and 41 this year. Indeed infestations designated as light were actually more common in 1977 than in 1978. The real differences lay in the percent that was heavily infested—less than 1% in 1977 as against 8% in 1978, and moderately infested, 2% as against 11% in 1978. (Heavy infestations were those with tassels nearly totally blackened, light were those with only exuviae or living aphids at points other than the tassel.) Richard Shade, who maintains Kentucky 27 as a baseline indicator of aphid pressure, had such high numbers this year that many tassels failed to develop at all (Pers. comm.). And a single cross, Nebraska 28 x B37, averaged 163.52/plant, about 3 times the 1977 level.

A cooperative federal and state survey indicates that the Hessian fly (*Mayetiola destructor* (Say)) infestations in wheat were at their highest level on record, both in terms of percentage infestation and in number of pupario present. Even the cultivars resistant to the prevalent race of flies (Biotype B) were infested at a higher rate (6.3%), compared with less than 1% over the last 6 years. Those lacking this factor suffered a 20.6% rate, up from 2.5% last year. Puparia/100 stems averaged 14.5 in all cultivars, 11 in resistant and 37 in non-resistant.

A combination of greenbug (*Schizaphis graminum* (Rondani)) and oat

bird-cherry aphid (*Rhopalosiphum padi* (Linnaeus)) was present in both wheat and oats, sometimes in large numbers and occasionally associated with barley yellow dwarf at least in the central districts.

The fall 1977 chinch bug (*Blissus leucopterus* (Say)) survey noted no infestations with potential economic consequences. Numbers in the EC side of the state have been increasing in the past years, and adults were observed in both August and September; their presence in August in the tassels and in September under leaf sheathes and husks of corn in that area was unusual.

Forage Legumes and Soybeans

The Mexican bean beetle (*Epilachna varivestis* Mulsant) was the only insect of significance in soybeans in 1978, and except for isolated fields in the SE and C districts, even it was of relatively little importance. This insect, present throughout the state on garden beans, is an economic pest of soybeans only south of Indianapolis and a few extensions further north (Parke and Wayne counties). A conspicuously infested field of soybeans was observed in Miami Co. this year. Such infestations so far north (as Clinton Co. in a previous year) have not persisted. And an infested field 11 miles from the Illinois line is the nearest to that state to date. The Wabash River has so far proven a barrier.

Egg numbers of the alfalfa weevil (*Hypera postica* (Gyllenhal)) in December of 1977 per 15 cm. sq. averaged about 10 in NW district, 30 in WC and C, and 60 in SW and SC district alfalfa, roughly comparable to fall 1975 numbers. The fall 1975 egg population hatched in the spring into a serious problem for 1976 spring alfalfa. But the long, hard winter of 1977-1978 took a heavy toll either by mechanical injury or reduced egg viability. Spring oviposition began early in April (Harrison Co.) and reached 80/15 cm. sq. by 17 Apr. when alfalfa averaged 20 cm. with 42% infestation rate and 2.1 larvae/infested stem. About a third of the S district fields had been treated by 1 May, and because rain and corn planting interfered with harvesting, more were treated later. North of US 50, and through the C districts, although there was often considerable damage, treatment of alfalfa weevil larvae was uncommon, and probably generally unwarranted. In LaPorte Co. (NW), treatment was recommended for 15 to 50 regularly surveyed fields; a series of very warm days accelerated weevil development and conspicuous damage occurred in a very short time. Northern districts generally escape serious economic damage from the alfalfa weevil.

Fall oviposition in 1978 was first observed during the first week of Oct. (Warren Co.) and remained at low (1-2/15 cm. sq.) levels through the north.

Of 50 alfalfa fields in LaPorte Co., 25 had to be treated (3rd cutting) for potato leafhopper (*Empoasca fabae* (Harris)) in addition to those that were cut early. About one-fifth of the same fields, regularly surveyed, needed treatment to prevent damage to the 4th cutting. A similar situation prevailed over most of the state, although similar data are not available.

Vegetables

(Vegetable summary provided by ALAN C. YORK)

Aphid populations increased to damaging levels considerably earlier than

usual causing problems on pepper and leafy greens in late June, July and into August. Also the Colorado potato beetle (*Leptinotarsa decemlineata* (Say)) seemed unusually active as first generation adults in the northern and southern regions of the state. Vincennes area tomato plants were seriously damaged by both adults and larvae. Record snow cover may have increased overwintering adult survival.

Striped- (*Acalymma vittata* (Fabricius)) and spotted- (*Diabrotica undecimpunctata howardi* Barber) cucumber beetles continued to be serious pests of melons in the SW portion of the state. Some fields in which controls were less than satisfactory had plant losses as high as 25-30%. Tomato fruitworm (*Heliothis zea* (Boddie)) showed up so near to the completion of harvesting that in few fields were controls applied. Cabbage looper (*Trichoplusia ni* (Hübner)) was present in large numbers on potatoes, tomatoes, cole and cantaloupe. Severe feeding was seen on the latter as well as the cosmetic problem of their pupation on the fruit surface. The same insect caused some concern in tomato canning factories for similar reasons; the insect apparently does not cause significant damage by foliage or fruit feeding, but pupation on the tomato fruit causes inspectors concern.

The European corn borer was a severe problem in sweet corn both by the 1st and 2nd broods in the central and northern parts of the state. Reports of corn borer damage to snap beans and peppers were not as numerous as in the past. Corn earworms caused some damage to sweet corn in the southern part of the state in the 1st generation. Numbers were usually low however in August and September when damage is normally the most severe.

Potato leafhopper was present in damaging numbers in potatoes and garden beans in most of the state. As a rule good control was achieved with conventional methods.

Ornamentals, Forest and Shade Trees

Leaf feeders, including the linden looper (*Erannis tiliaria* (Harris)), fall cankerworm (*Alsophila pometaria* (Harris)), greenstriped mapleworm (*Dryocampa rubicunda* (Fabricius)) and several other species were especially abundant and destructive in trees south of Indianapolis. Bagworm (*Thyridopteryx ephemeraeformis* (Haworth)) was abundant in the same area. The forest tent caterpillar (*Malacosoma disstria* Hübner) which has been a serious pest in the past years, waned in numbers.

The most serious pest of lawns was the northern masked chafer (*Cyclocephala borealis* Arrow); larvae were also sometimes quite numerous in weedy cornfields, trashy gardens and the like in the late fall.

Man and Animals

The extension service received fewer inquiries concerning household pests than even last year. The Indian meal moth (*Plodia interpunctella* (Hübner)) was the most commonly reported stored grain pest; the foreign grain beetle (*Ahasverus advena* (Waltl)) remains high on the list and is the most commonly noted by pest control professionals. A heavy flight of pavement ants

(*Tetramorium caespitum* (Linnaeus)) was reported from Indianapolis on 24 July. And the carpenter bee (*Xylocopa virginica* (Linnaeus)) was more numerous there this year as well; it was often reported from the rest of the state, too. Another hymenopteran, *Vespula germanica* (Fabricius), is gaining prominence. By mid-November, only 2 cases each of LaCrosse encephalitis and St. Louis encephalitis had been confirmed for the state.

Beneficial Insects

The ratios (in % of total of these 4 species) between the spotted (*Coleomegilla maculata* DeGeer), the convergent (*Hippodamia convergens* Guerin-Meneville), the 13-spotted (*Hippodamia tredecimpunctata* (Say)) and *Cycloneda sanguinea* (Linnaeus) lady beetles as indicated by sticky trap collections in a Tippecanoe Co. corn field follow: 69:6:16:9. In 1977 ratios were 27:65:3:5. In numbers there was a three-fold increase in these traps as well as in cornfields through the state.

Parasitism of the alfalfa weevil by *Bathyplectes anurus* (Thomson) and *B. curculionis* (Thomson) together averaged 23% in the state in collections made between 15 May and 6 June. *B. anurus* was an important constituent of the parasite population in only 2 of the counties surveyed, Harrison and Washington. The WC district had the highest rate of parasitism (43%) followed by the NW and NC with about 25% each.

Mating Behavior of Butterflies (Papilionoidea) and Skippers (Hesperioidae) in Indiana

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Introduction

From the spring of 1968 until the fall of 1978 the author researched the mating behavior of the butterflies (Papilionoidea) and the skippers (Hesperioidae) found *in copula* in Indiana. The remaining Lepidoptera have been excluded. In all, 719 pairs were collected *in copula*, representing 45 species. Slightly over 50 percent of the mating pairs comprise one species (*Pieris rapae*), the common European Cabbage White. Three pairs were crossbreeding.

The families included in this paper are the Danaidae, Satyridae, Nymphalidae, Lycaenidae, Pieridae, Papilionidae, and Hesperiidae. Three families—Libytheidae, Riodinidae, and Liphyridae—have not been included because not one of the five Indiana species in these three families has been found *in copula* in Indiana.

The mating date, the time of mating, the temperature at the time of mating, the active sex partner (the sex which carries its partner in flight when *in copula*), nuptial and courtship behavior are noted. All recorded times for mating have been converted to Eastern Standard Time (E.S.T.). The nomenclature follows Howe (2).

Part One: Mating Behavior of Butterflies (Papilionoidea)

Danaidae

Danaus plexippus plexippus (Linnaeus). Forty-five mating pairs. The male was always the active flight partner; however, Pronin (4) reported the female as the active partner. His record is doubtful.

With the exception of two pairs mating in June (29. VI. 1977) the other 43 pairs mated in July and August. One pair mated 22. VIII. 1977, 9:45 A.M., at a cold 59° F, Silver Lake, Kosciusko Co. The male could not fly when released. Its wings were spread open toward the sun, a process of thermoregulation prior to morning flight. The other pairs were mating between 70° F and 92° F, the great majority mating in the mid-70s and mid-80s. Mating usually takes place from late morning to late evening (11:00 A.M.-6:45 P.M.), the greatest number mating between 2:00 P.M. and 4:30 P.M. A few pairs may remain *in copula* throughout the night and the author has seen mating pairs resting in the tree-tops at sunset.

Mating *plexippus* have been collected in Wabash, Kosciusko, Randolph, Delaware, Allen, and LaGrange counties. Three to four broods.

Satyridae

The author collected three species of this family *in copula*: *Lethe eurydice eurydice* (Johansson). Recently *L. eurydice* and *L. appalachia* have been separated as distinct species (no longer *Lethe eurydice appalachia* in Indiana) (5). Both species occur in Indiana. On 29. VII. 1972 pair of *L. e eurydice*, 12:30 P.M., 68° F, in a woodland bog, North Manchester, Wabash Co. Female was the active flight partner. One brood.

Eupychia cymela cymela (Cramer). Five pairs from June 6, 1969 to July 4, 1978. Mating times were from 11:50 A.M. to 3:10 P.M. The female was always the active flight partner. Temperatures, not always recorded, were generally in the 70s. Mating pairs from Wabash, Kosciusko, and LaGrange counties. One brood.

Cercyonis pegala (Fabricius). Thirty-four pairs *in copula*. In northeastern Indiana, mating of *pegala* and its many subspecies or integrating forms (*olympus*, *alope*, *nephele*, and others) begins in late July and has continued until August 25 (a late date), reaching its peak around mid-August. Pairs have been collected as early as 9:05 A.M. and as late as 5:20 P.M. Normally mating occurred between 11:00 A.M. and 3:00 P.M. Temperatures ranged from 65° F to 80° F, but the 70s and 80s dominated.

So far *C. pegala* has not been found in southern Indiana, mating or otherwise. When found *in copula* in Wabash and Kosciusko counties, the female was the active flight partner. One brood.

Nymphalidae

According to Miller and Clench (3), there is much variety in this family in the sex of the active flight partner; it may be the male of some species, the female of others, or the sex roles may alternate between the partners. However, the females clearly predominate. In the nine mating species of nymphalids collected in Indiana the author has found females to be the active partners.

Limenitis astyanax (Fabricius). Two pairs: 21. VIII. 1969, 3:45 P.M., Silver Lake, Kosciusko Co. and 7. VI. 1970, 2:25 P.M., Brown County State Park. When the last pair was disturbed, it flew into a tree at the edge of the meadow. The female carried the limp male. Three broods.

Limenitis archippus archippus (Cramer). Seven pairs found breeding in July, August, and September. The lowest mating temperature was 70° F and highest 90° F. Mating occurred in the afternoons from 1:00 P.M. to 4:00 P.M. Copulating pairs were collected in Wabash, Kosciusko, and Carroll counties. The female was always the active flight partner. Two broods.

On 7. IX. 1971, 1:30 P.M. the author witnessed a *L. archippus* x *L. astyanax* *in copula*, North Manchester, Wabash Co., but was unable to catch this wild mating pair because of a dangerous bull protecting his turf. These two species have been known to hybridize.

Precis coenia (Hübner). Two pairs: 30. VII. 1970, 3:05 P.M., in a cow pasture, Delphi, Carroll Co.; and 31. VIII. 1970, 9:50 A.M., 60° North Manchester, Wabash Co. When disturbed both pairs settled again in the deep grass. The females were the active flight partners. One or two broods.

Phyciodes tharos tharos (Drury). Fifty-eight mating pairs. Found in copula every month from May 17 until October 7 (a late date for any species). Mating sometimes occurs in the early morning hours (9:00-10:00); however, the great majority mated between 11:00 A.M. and 3:30 P.M. Only a few mated between 3:30 and 5:45 P.M.

In one pair, courtship lasted only five minutes prior to copulation. Twice the female flew to a new location only to be pursued by the male. When the pair perched on a blade of grass, there were moments of wings rubbing and antennae touching. Then their caudal portions were joined achieving copulation. When disturbed the female, as is always the case in this species, flew a few feet carrying the male which had its wings completely closed. The author watched one pair courting for one and one half hours but no mating occurred.

Temperatures for mating ranged from 66° F to 90° F; however, the mid-70s to mid-80s accounted for the majority. Mating occurs in a variety of habitats—near margins of lake, rivers, and streams, occasionally in an open lawn or field. Mating pairs were collected in Wabash, Kosciusko, Carroll, and LaGrange counties. Four, possibly five broods.

Chlosyne nycteis nycteis (Doubleday). Three pairs: 17. VI. 1971, 3:05 P.M., 85° F, Milford, Kosciusko Co.; and 28. VI. 1978, 3:30 P.M., 88° F, Silver Lake, Kosciusko Co. The first pair mated in a raspberry patch surrounded by trees, the last pair rested on a dogbane shrub. The female was the active flight partner. One brood.

Euphydryas phaeton (Drury). One pair: 15. VI. 1977, 12:20 P.M., 72° F, North Manchester, Wabash Co. The pair was copulating on a shrub in a woodland marsh. After photographing them, the pair was captured in the author's net. The female, carrying the male, flew up and down in the white bag, a good technique for determining the active sex partner. One brood.

Vanessa (Cynthia) virginianensis (Drury). One pair: 10. VII. 1977, 3:20 P.M., 80° F, in alfalfa/red clover field, Silver Lake, Kosciusko Co. The female was the active flight partner. Two broods.

Speyeria cybele cybele (Fabricius). Ten mating pairs. With the exception of one copulating pair (9. VII. 1969), the other pairs mated in late June. Six pairs were mating 22. VI. 1977, 70° F, between 2:15 P.M. and 3:25 P.M., Silver Lake, Kosciusko Co. They were all mating near the border of a forest and grass field. Two other pairs mated when the temperature was 84° F.

On 12. VII. 1978 the author observed a male *cybele* pursue a female. The female seemed to reject the pursuer by positioning herself on the underside of a blade of grass with the male directly above on the upper surface. She remained motionless with her wings closed, as the helpless male fluttered his wings and moved his antennae up and down. He soon left her and pursued another female, again being rejected in the same manner. The females were the active flight partners. One brood.

Boloria bellona (Fabricius). One pair: 30. IX. 1975, 3:45 P.M., 72° F, North Manchester, Wabash Co. The pair settled in a grassy field. For hours the author has watched this abundant species engage in its courtship activities, but have not

yet figured out the rejection method used by the females. The female was the active flight partner. Two broods.

Lycaenidae

The author supports writers Miller and Clench that "all the reliable records indicate that the female is the active partner in this family" (3). To date the author has collected only six lycaenid species of this large family *in copula*.

Satyrium caryaevorus (McDunnough). One pair on dogbane, edge of a forest, 26. VI. 1977, 3:50 P.M., 83° F, North Manchester, Wabash Co. One brood.

Satyrium acadica acadica (W. H. Edwards). One pair: 10. VI. 1977, 10:45 A.M., 60° F, settled in a patch of sedge grass east of Lake Waubee, Milford, Kosciusko Co. Dogbane nearby is one of its favorite host plants. One brood.

Lycaena (Tharsalea) hyllus (Cramer). Three pairs: 10. IX. 1969, 12:15 P.M., 62° F; 9. VII. 1970, 2:15 P.M., 80° F; and 10. VI. 1973, 5:35 P.M., 85° F—all in North Manchester, Wabash Co. and all breeding among *Iris versicolor* in marshy fields. Two broods.

Lycaena phlaeas americana (Harris). Four pairs: 21. IX. 1968, 8:45 A.M., 50° F, an extremely low temperature for mating, North Manchester, Wabash Co., indicating that the pairs had copulated through the night; 30. VIII. 1969, 10:30 A.M. and 7. IC. 1969, 11:40 A.M., both in North Manchester. The fourth pair matched 29. V. 1972, 9:05 A.M., Mongo, LaGrange Co. It is curious that no pairs of this species were found mating in the afternoons. Two broods.

Celastrina argiolus pseudargiolus (Boisduval & LeConte). One pair: 23. VI. 1969, 2:30 P.M., North Manchester, Wabash Co. Although this species with its spring and summer forms flies from mid-April until mid-September, its courtship and breeding habits seem to defy observation. Three broods.

Everes comyntas comyntas (Godart). Twelve pairs, the mating period extending from June 24 until October 7 (rarely). Mating usually occurs from 1:45 P.M. to 6:20 P.M., however, one pair was mating (2 IX. 1970) at 10:35 A.M., 70° F, Silver Lake, Kosciusko Co., on a *Polygonum* leaf. Mating temperatures were primarily in the 70s and 80s. Look for them in or near wooded areas. One brood.

Pieridae

In this family the author has found the male of each species observed *in copula* to be the active flight partner.

Pieris protodice protodice (Boisduval & LeConte). One pair: 24. VIII. 1971, 11:15 A.M., 65° F, Mongo, LaGrange Co. When the female wants to reject the approaches of the male, she does so by raising her abdomen to an almost perpendicular angle with her own thorax and vibrates her abdomen. Or, she may simply fly high into the air with the male frequently bumping her with his wings until they finally fly apart, "falling" to the ground well apart from each other.

Pieris napi oleracea (Harris). Four pairs, all *in copula* near watercress *Nasturtium officinale*, one of its larval foodplants, in the largest tamarak bog of

Indiana, Pigeon River Fish and Wildlife Area, Mongo, LaGrange Co. The first pair (23. VII. 1975) was mating at 12:35 P.M., 90° F (6). On 29. IV. 1978, 11:00 A.M., 70° F, the heavily veined spring form was *in copula* on the leaf of a Marsh Marigold amidst the usual watercress. Two pairs, 11. VII. 1978, were found at 2:25 P.M and 2:40 P.M., the temperature reading 73° F for both pairs. Three broods.

Pieris rapae (Linnaeus). Space will not permit a detailed record of the 363 pairs found *in copula*. Only a summary of the most relevant data will be presented. Mating takes place from early morning until late in the evening, some mating as early as 9:30 A.M. and others as late as 6:30 P.M., a few probably remaining *in copula* throughout the night. More observations of this phenomenon are needed.

Pierids, like most other butterflies, generally mate on warm, humid days, periods free from strong winds but not necessarily free from gentle rain followed by periods of sunshine. In the main, mating occurs from 11:00 A.M. until 4:00 P.M. Temperatures vary from a low 64° F (rare) to a high 91° F; however, the mid-70s to mid-80s account for the vast majority. The Cabbage White may be found copulating almost anywhere, even while crossing a major highway. Alfalfa fields, clover fields, and gardens where cabbages and other Cruciferae (larval foodplants) grow, supply the greatest number of breeding pairs.

Numerous observations show that the female, unprepared for copulation, will raise its abdomen nearly upright above its opened wings and vibrate its abdomen and antennae, a proven rejection method. After a full minute or two the male will fly away to pursue the first female scented. A mating pairs may be bombarded by five or more males, but the author has never seen the copulating pair separated by the additional male activity.

Although entomologist report only three broods, may data show that in 1969 there were five broods in northeastern Indiana: (1) April 23 to May 6, (2) June 16 to July 1, (3) July 12 to August 4, (4) August 19 to September 5, and (5) September 14 to 22. This fact should not go unnoticed for this serious European pest and it is found breeding in Wabash, Kosciusko, Allen, Stuben, LaGrange, Elkhart, Noble, Delaware, Randolph, and Brown counties. Three to five broods.

Colias eurytheme and the next species (*Colias philodice*) occasionally hybridize. But, from 1964 until 1978 only two pairs have been found crossbreeding, even though both species fly together in the same hayfields, *philodice* doing best on clovers (*Trifolium*) and *eurytheme* being more successful on alfalfa (*Medicago sativa*). On 5. IX. 1969, a female *C. eurytheme* was found mating with a male *C. philodice*, 1:05 P.M., Silver Lake, Kosciusko Co. The other pair was collected 24. VIII. 1977, 1:25 P.M., 64° F, Silver Lake, with the male being a *philodice*. Although some hybridization takes place between these two species, it is best for the present to treat them as two separate species.

Only one male of *C. eurytheme* was found breeding a white or "alba" form female. Quite a contrast to the next species (*C. philodice*), where 40 percent of the females were "alba" forms. The rejection method of this species is the same as

used by the other pierids. Mating pairs have been collected in Wabash and Kosciusko counties.

Colias philodice philodice (Godart). Ninety-seven pairs. As in *P. rapae*, the information is overwhelming and difficult to analyze. Only a few pairs were matching before 11:00 A.M., the vast majority mating between noon and 4:00 P.M. A few, however, have mated as late as 6:35 P.M. The coldest mating temperature was 60°F, but most occurred in the higher 70s and 80s. A lot of courtship activity begins when the temperature reaches 68°F. During August and September mating activity reaches its peak, but it may occur any month from mid-June until early October.

It is hard to explain genetically why 40 percent of the females found *in copula* were "alba." The percentage seems too high. This matter needs more research.

Mating pairs have been found in Allen, Wabash, Kosciusko, Huntington, Elkhart, Carroll, and LaGrange counties. Two broods.

Papilionidae

Very little is known with certainty about the mating of swallowtails in any part of the world. Pronin (4) reported *Papilio machaon* Linnaeus in Europe (1:00-2:00 P.M. with the female as the active flight partner) and *Papilio multicaudatus* Kirby (California, 2:00-3:00 P.M.) without designating the active flight partner. Clench found the male *Battus montezuma* to be the active partner (personal correspondence). It is odd that species in this family are so difficult to find *in copula*. The author tried releasing several black female *Papilio glaucus glaucus* (Linnaeus) where many yellow males were flying in the tree-tops. Whenever a male detected the female, he would pursue her but no mating took place under these circumstances. Four Indiana species have been collected *in copula*, but my data are inconclusive.

Papilio philenor philenor (Linnaeus). One pair: 23. IV. 1976, 2:50 P.M., 70°F, Silver Lake, Kosciusko Co.; settled on a branch of a Hawthorne tree. The female was the active flight partner. Two broods.

Papilio polyxenes asterius (Stoll). One pair was reported to the author by the author's daughter (biology minor in college): 5. VII. 1978, 3:30 P.M., Daleville, Delaware Co. Unfortunately the active sex partner was not noted. Two or more broods.

Papilio troilus troilus (Linnaeus). One pair: 17. VII. 1971, 1:15 P.M., 76°F, Silver Lake, Kosciusko Co., mating in a woodland raspberry patch. After chasing the disturbed pair for nearly 100 yards through the woods and its heavy underbrush, the pair settled on a low bush. After netting them, the author determined that the female was the active flight partner; however, sex identification in these crowded quarters was difficult. Two broods.

Graphium marcellus (Cramer). Two pairs: 16. VI. 1968, 9:00 P.M. and 19. V. 1978, 1:45 P.M., 82°F—both in a woods, Silver Lake, Kosciusko Co. The flight partner of the first pair could not be determined because the pair took refuge in a raspberry patch. Two broods, possibly three.

When wanting to mate the males of two species—*Graphium marcellus* and *Papilio glaucus*—try to force the females to the ground, fluttering over them until rejection has been accepted.

Part Two: Mating Behavior of Skippers (Hesperioidae)

Hesperiidae

Seventeen species of this large family *in copula*, but the data is usually limited to a single pair or to a few pairs of each species. In every case the female was the active flight partner.

Euphyes conspicus conspicus (Edwards). One pair: 3. VII. 1970, 1:35 P.M., North Manchester, Wabash Co., mating among the sedges of a woodland marsh. One brood.

Euphyes bimaculata (Grote & Robinson). One pair: 20. VI. 1969, 1:00 P.M., North Manchester, Wabash Co., in a marshy meadow. One brood.

Euphyes vestris metacomet (Harris). One pair: 20. VI. 1969, 2:00 P.M., Silver Lake, Kosciusko Co., in woods copulating on a raspberry twig. One brood.

Atrytone delaware delaware (Edwards). One pair: 29. VI. 1970, 3:30 P.M., 89°F, in a grassy field, Silver Lake, Kosciusko Co. One brood.

Atalopedes campestris (Boisduval). One pair: 6. VIII. 1971, 3:15 P.M., 78°F, resting on *Helianthus strumesus*, a sunflower in a marshy field, North Manchester, Wabash Co. One brood.

Wallengrenia otho egeremet (Scudder). One pair: 9. VIII. 1971, 12:15 P.M., 79°F with high humidity, North Manchester, Wabash Co., on dogbane flowers. Two broods.

Polites coras (Cramer). Four pairs: 6. VI. 1970, 10:55 A.M., Tippecanoe Co.; 10. VII. 1971, 9:15 A.M., 76°F, North Manchester, Wabash Co.; 18. VIII. 1971, 3:30 P.M., 90°F, North Manchester; and 24. V. 1977, 12:55 P.M., 84°F, North Manchester. This last pair copulated on a blade of cattail in a marshy field. Two broods.

Polites themistocles (Latreille). Three pairs: 20. VI. 1969, 1:25 P.M., Silver Lake, Kosciusko Co.; 20. VI. 1971, 1:45 P.M., 85°F, North Manchester, and 24. VI. 1971, 2:00 P.M., 85°F, North Manchester, Wabash Co. One brood.

Polites origines origines (Fabricius). Three pairs: 10. VII. 1969, 3:30 P.M., Silver Lake, Kosciusko Co.; 11. VII. 1969, 3:10 P.M., Silver Lake, Kosciusko Co.; and 6. VI. 1970, 10:55 A.M., Tippecanoe Co. One brood.

Polites mystic (Edwards). One pair: 30. V. 1977, 4:55 P.M., 80°F, North Manchester, Wabash Co., mating on a blade of marsh grass in an open field. One brood, possibly two.

Thymelicus lineola (Ochsenheimer). Four pairs: 20. VI. 1969, 2:25 P.M., Silver Lake, Kosciusko Co.; 27. VI. 1970, 11:45 A.M., Mongo, LaGrange Co.; 26. VI. 1971, 1:00 P.M., 84°F, Silver Lake, Kosciusko Co.; and 3. VI. 1977, 11:00 A.M., 70°F, Silver Lake, on alfalfa. One brood.

Ancyloxipha numitor (Fabricius). Three pairs: 22. IX. 1969, 1:25 P.M.,

71° F, Silver Lake, Kosciusko Co., in grass by the border of a pond; 7. X. 1970, 2:15 P.M., 90° F, Carroll Co., in a grassy field; and 24. V. 1977, 9:20 A.M., 80° F, North Manchester, Wabash Co., resting on a blade of grassy in a marshy field. Two broods.

Pholisora catullus (Fabricius). Three pairs: 25. VII. 1969, 2:30 P.M., Silver Lake; 4. X. 1970, 12:40 P.M., 78° F, Silver Lake; and 16. VII. 1971, 12:45 P.M., 80° F, Silver Lake, Kosciusko Co.—all in an alfalfa field. Two broods.

Erynnis horatius (Scudder & Burgess). One pair: 30. IV. 1977, 1:45 P.M., 65° F, Silver Lake, Kosciusko Co., mating near the top of a dead raspberry twig where the mimicry was nearly perfect. Two broods.

Erynnis juvenalis juvenalis (Fabricius). Two pairs: 30. IV. 1976, 2:00 P.M., 65° F, Silver Lake, Kosciusko Co.; and 25. V. 1978, 11:40 A.M., 78° F, Mongo, LaGrange Co. The courtship of this last pair took less than one minute. The pair settled on a twig of a bush, turned their caudal portion toward each other and joined in copulation. One to two broods.

Thorybes pylades (Scudder). Five pairs: 11. VII. 1969, 3:10 P.M., Silver Lake; two pairs 1:20 P.M. and 2:25 P.M., Silver Lake, both pairs mating on a small path in a woodland raspberry patch; 23. VI. 1970, 2:35 P.M. and 25. VI. 1970, two more pairs mating on the ground in the above area, Silver Lake, Kosciusko Co. One to two broods.

Epargyreus clarus clarus (Cramer). One pair: 15. VIII. 1970, 10:40 A.M., 72° F, North Manchester, Wabash Co. One brood.

Tentative Conclusions and Research Questions

In the Danaidae and Pieridae families, the male of each species collected *in copula* was the active flight partner. In the Satyridae, Lycaenidae, and Hesperidae families, it was always the female which carried the male in flight. In the Nymphalidae and Papilionidae families, there was a variety of flight patterns, sometimes the male but more often the female being the active flight partner. Indiana records of copulation are needed for the Libytheidae, Riodinidae, and Liphyridae families.

More observations of the female's rejection techniques would be interesting for the remaining species. Only the pierids, and possibly that of one nymphalid (*Speyeria cybele*), are partially understood.

Many questions remain to be researched: How long do the various species remain *in copula*? How often will the same female or male mate? Why do so many common species escape observation when they are copulating? What is the purpose of nuptial flights high into the sky? Which species hybridize? More data are needed to answer these and other intriguing questions.

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Distribution, Abundance, and Seasonality of Tiger Beetles (Cicindelidae) in the Indiana Dunes Region

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Introduction

The sandy areas around the southern end of Lake Michigan provide particularly favorable habitats for various species of tiger beetles. In the early 1900's, V. E. Shelford conducted studies of cicindelids in this area and contributed much to the knowledge of the ecology of this interesting group of insects. His studies are, in fact, among the few significant ecological studies of tiger beetles in the United States. He detailed in laboratory and field studies the varied life history patterns and larval characteristics of the 12 species he found in this area (4). In another "classic" work he showed how soil conditions were important in limiting the distribution and abundance of tiger beetle species and, in particular, how the adult female of a species selects oviposition sites and thus provides optimum conditions for survival and development of the larvae of that species (5). This paper included the formulation of his well known "law of toleration" which was based on studies of tiger beetles. Earlier he demonstrated the relationship between the distribution of various species and the successional stage of the habitat (3).

Shelford's work was done primarily in the southeastern part of Illinois where a somewhat different composition of species are present compared to what I found in northwestern Indiana. For example, 4 species he found commonly there were not found during my study and probably do not exist in the Indiana dunes region. Also, in the time since his work the natural environment of the area has changed radically, resulting in an apparent reduction in the distribution and abundance of most species. This study is part of a continuing project aimed at determining distribution, abundance, seasonality, and habitat ecology of tiger beetles in Indiana.

Methods

I conducted the study with the assistance of several Franklin College students from May 1977 through October 1978. Observations were made on 23 different dates during this period. Much of northwestern Indiana was surveyed for tiger beetle habitats, but the most favorable, most accessible, and least disturbed sites were found in the Indiana Dunes State Park and National Lakeshore or in adjacent areas. Only these sites, located between Gary and Michigan City, are included as part of this study (Fig. 1). A variety of habitat and ecological community types were identified and many had at least 1 species of tiger beetle.

Since tiger beetles are very active, wary insects which fly off at the slightest movement they are difficult to sample. Abundance and density data were

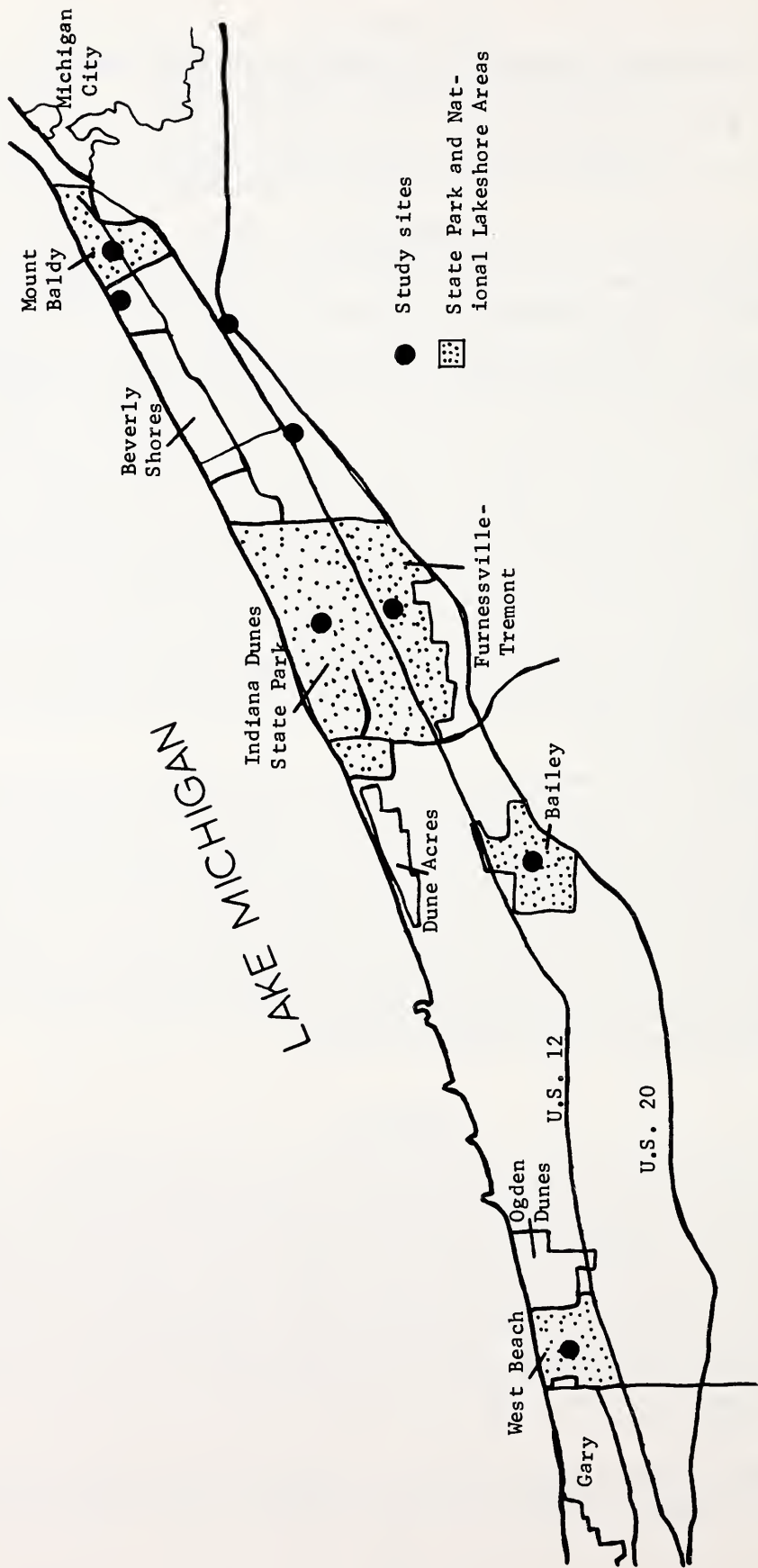


FIGURE 1. Map of the Indiana Lakeshore area showing primary study sites.

obtained using a direct count method (6). Two investigators would walk together slowly in a straight line and examine the ground 5-10 meters ahead. All beetles within about a 4 meter wide area were readily observed, then identified and recorded. The total area covered in each habitat or site was estimated so that densities could be determined. There was considerable variation in the total area examined in each site. Occasional specimens were collected to confirm identifications and for a reference collection. Additional observations provided information on cicindelid activity and behavior. Although this method of survey is rather simple, it was appropriate for giving comparative abundance and density for the different species.

Results and Discussion

Distribution and Habitats. Eight species of tiger beetles and 12 types of habitats were found (Table I). *C. macra*, *C. repanda*, and *C. hirticollis* preferred fluvial habitats, specifically the edges of lakes, ponds, or streams. The open sandy flats, slopes and dune habitats were preferred by *C. lepida*, *C. formosa*, *C. scrutellaris*, and *C. punctulata*. *C. sexguttata* was found only in forest sites.

Cicindela macra Lec. was mostly restricted to the edge of Lake Michigan, where large numbers occurred on damp sand within 5 meters of the water edge. Occasionally individuals were on the middle beach and on the foredune faces. Small numbers were also seen at night resting on debris along the beach and on herbaceous vegetation at the base of the foredunes. Most other collection records for this species in Indiana are from the Lake Michigan beaches.

Cicindela repanda Dej. is an extremely common and widespread species in the United States. I have found it in all Indiana counties where I have sampled. Populations were found along the Lake edge and along several interdunal ponds in association with *C. hirticollis*. Small numbers were present in many of the other habitats, typifying this species wide distribution.

Cicindela hirticollis say prefers fluvial habitats where sandy beaches or flats are present near water. Several populations were found along the edges of several interdunal ponds and one blowout pond. Both ponds had permanent water available at or near the surface, even during late summer droughts. Occasional individuals were seen in several of the adjacent habitat types.

Cicindela lepida Dej. occurs over much of the United States, but is apparently restricted to areas with well developed sandy soils. Its distribution and abundance in the dunes and in other parts of its range seem to be decreasing in recent years. Preferred habitats in the dunes region were blowouts, the back beach, and foredune faces, but individuals were sometimes seen along the lake edge. Twice small numbers of adults were found in shallow burrows. Once was at mid-day when surface temperatures of the sand were very high and on another day during mid-morning after a cool rain shower. Adult cicindelids have been reported to dig such temporary burrows when conditions are unfavorable (7). This behavior would be especially important for a species like *C. lepida* which prefers the open sand flats where surface temperatures are frequently quite high.

Cicindela formosa say occurs over many of the sandy regions of Indiana, especially along the major rivers and in the northern part of the State. In this

TABLE I *The numbers of sites in each habitat type where species occurred.*

Species	Lake edge	Stream edge	Interdunal pond	Blow-out pond	Damp depression	Back beach	Fore dune face	Fore dune ridge	Blow-out	Dune flat, slope	Non-dune flat, slope	Woods trail
<i>C. macra</i>	8*					4	3					
<i>C. repanda</i>	5*	1	5*	4	2	4					2	
<i>C. hirticollis</i>	4		4*	1		3						
<i>C. lepida</i>	4					5*	4*		4*		1	
<i>C. formosa</i>	1		1			3	3*	8*	6*	5*	3	
<i>C. scutellaris</i>						1		3		5*	3	
<i>C. sexguttata</i>												3
<i>C. punctulata</i>	1					1		1	2	1	1	1

*Habitats where species were most abundant

study it was found in more types of habitats (8) than any other species. It preferred foredune ridges, blowouts, and dune flats and slopes. Small numbers could also be found in most any dunes area that was open and sandy.

Cicindela scutellaris say is associated with *C. formosa* in sandy habitats over much of the United States. It was found in the above habitat types where *C. formosa* was abundant, except for blowouts. It was also more restricted in its habitat distribution though, apparently preferring more stabilized sandy areas where vegetation was not as sparse.

Cicindela sexguttata Fab. is the common six-spotted tiger beetle which is widely distributed along dirt paths, roads, and open areas in forests. Like *C. punctulata*, it was not common during this study and occurred in only 3 sites, all established park trails through fairly mature forests. Its limited occurrence is probably due to its preference for mesic woodlands rather than the xeric pine-oak forests in the dunes region.

Cicindela punctulata Fab. is an extremely common species in Indiana where it can be found in a great variety of sites with bare soil, such as paths, roads, eroded patches or field edges, agricultural areas and even pavements. This species was, however, not common in the dunes region. Only single individuals were seen but in a variety of habitats.

Abundance and Density. Maximum abundance and density were determined for each species and are given in (Fig. 2). These values are the highest

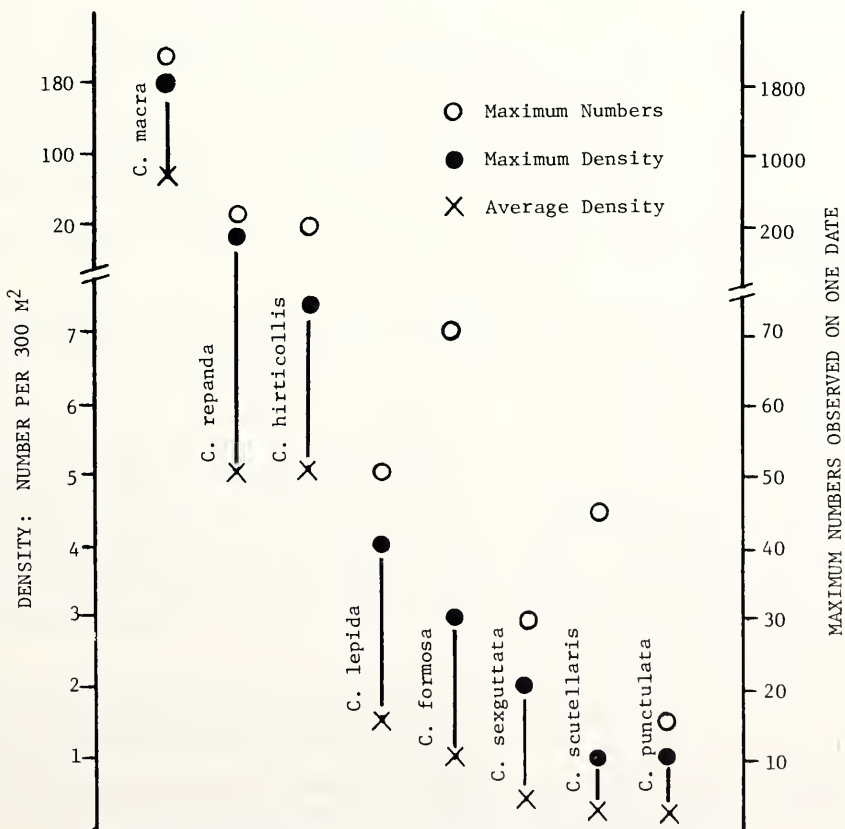


FIGURE 2. Densities and maximum numbers observed for each species. Maxima are for one site on one date.

recorded at all sites during the 2 years. Average densities are based on counts at all sites where a species occurred, but only on dates during the optimum season. In general, the species can be grouped into 3 levels of density and abundance. *C. macra* was very abundant and dense relative to the other species. On one date nearly 2000 were seen along several hundred meters of lake edge, and frequently hundreds were present in localized lengths of beach. Densities were also high and commonly 50-100 beetles were present in a 300 square meter area. They literally swarmed along the water edge and often over half were *in copula*.

A lower level of density and abundance was seen for *C. repanda* and *C. hirticollis*. On several dates 100-200 individuals of each species were seen with densities of 10-20. In addition to being less abundant and dense these species were more scattered within a habitat than was *C. macra*.

The other 5 species varied some in abundance but densities were always less than 5. *C. lepida*, *C. formosa*, and *C. sexguttata* were often present in small localized populations of 5-15 individuals, but *C. scutellaris* and *C. punctulata* were always scattered such that rarely were more than 2 or 3 collected in one area.

It is significant that the 3 most abundant species occurred in the fluvial habitats which are of rather limited occurrence in the dunes region. The other non-fluvial species occurred in habitats that were more extensive, but individuals were more sparse within those habitats.

Seasonality. Three seasonal patterns for tiger beetles have been recognized by Shelford (4). Species are designated as spring, spring-fall, or summer depending on the time of the year that the adults are active. All 3 patterns are represented by the dunes species and are given in (Fig. 3). The graphs for each species were constructed primarily from observations during this study, but in part from information I have obtained in other areas of Indiana. Times of adult emergence can be quite accurately determined, often to the day, because tenerals have a characteristic soft, wet appearance.

C. sexguttata was the only spring species. Adults start emerging in mid-April and by early May the population peaks but declines in late June when adults begin to die off.

C. macra, *C. lepida*, and *C. punctulata* are typical summer species with similar patterns of adult activity. *C. lepida* emerges somewhat later than the others while some individuals of *C. macra* survive longer and can be found throughout September. *C. hirticollis* is unusual in that adults are active from May through October. Shelford (4) found this was because a second brood of adults emerges around mid-July before those individuals which emerged in spring have died off.

C. repanda, *C. formosa*, and *C. scutellaris* are spring-fall species and show similar seasonal patterns. Adults emerge from hibernation in April, reach peak numbers in several weeks when all have emerged, and then begin to die off by early July. Some individuals of all 3 species can be found throughout the summer, with *C. formosa* being most common. In late August a new brood of adults emerges from pupae that developed during late summer. These are active

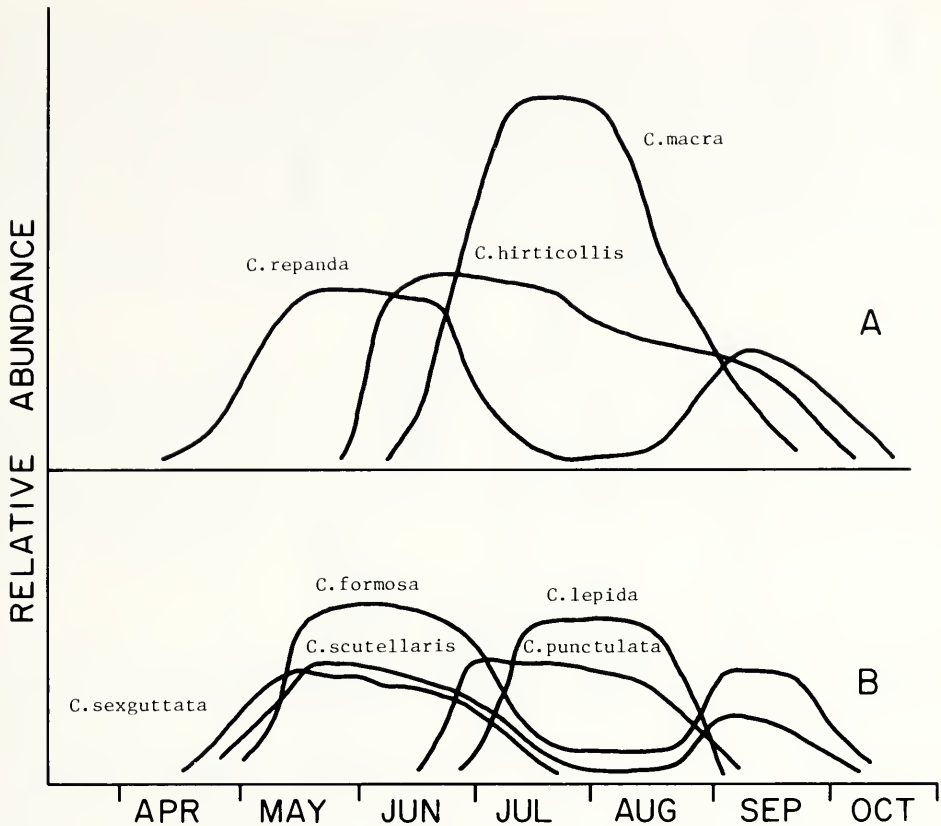


FIGURE 3. Relative abundance of fluvial (A) and nonfluvial (B) species throughout the year. Constructed from 1977 and 1978 data.

for much of September and October, yet are apparently sexually immature when they dig burrows for hibernation.

Spatial and Temporal Segregation. The 7 species inhabiting beaches and dunes of Lake Michigan show rather distinct spatial and temporal partitioning of these habitats. Most species were well segregated by their habitat preferences as can be seen in (Fig. 4). Both *C. macra* and *C. repanda* prefer the lake edge but are clearly segregated seasonally. *C. repanda* also is found along the interdunal pond edges with *C. hirticollis*, but the latter is most abundant during summer when *C. repanda* is scarce. *C. formosa* and *C. scutellaris* are both spring-fall species and both occur commonly on dune slopes and flats. However, *C. scutellaris* was found in sites that were more stabilized and with more vegetation cover. *C. lepida* is also in these same habitats but is a summer species and clearly segregated seasonally. *C. punctulata* was most often found on the dune flats and slopes farther from the water than the above species, but in the same types of habitats. Although the spatial and temporal segregation observed for adults is quite marked, Shelford (4, 5) has reported that larvae of some of these species are found in different microhabitats also.

Other Dune Species. A search of the literature and of museum records indicates that 5 other species have been collected from the dunes areas of Indiana and Illinois. Shelford (4) reported *C. tranquebarica*, *C. purpurea*, and *C. duodecimguttata* from the Illinois dunes area north of Chicago, and he worked

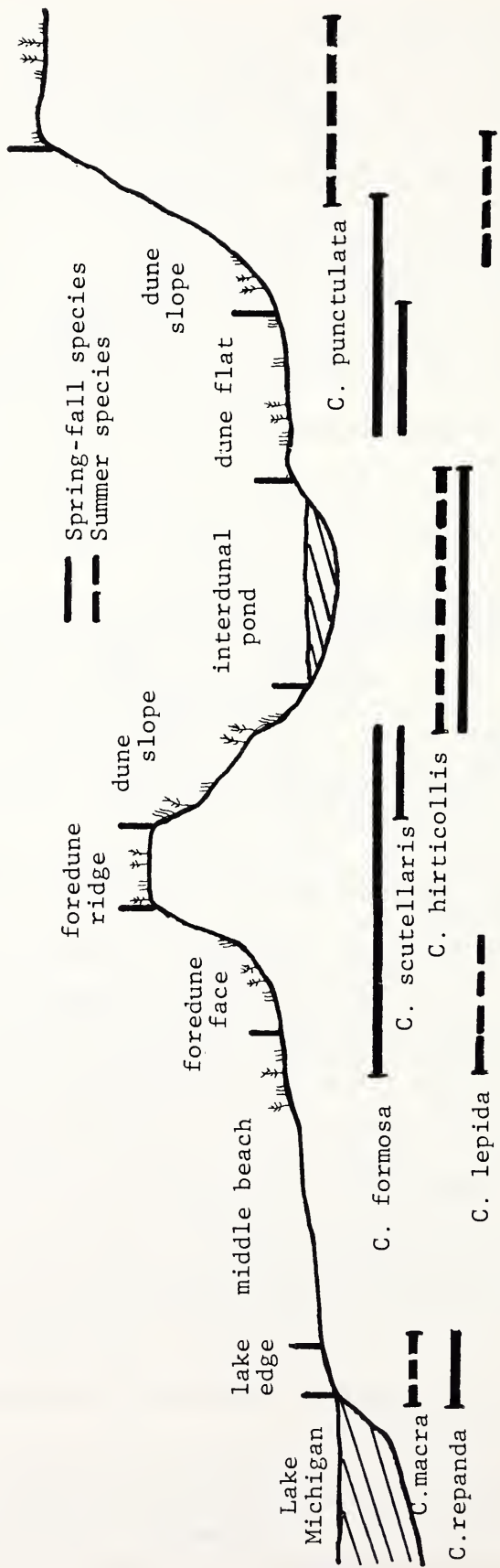


FIGURE 4. Profile of the Lake Michigan area showing habitat preferences and seasonal patterns of tiger beetle species.

out life histories for these species there. I have recently collected *C. duodecimguttata* from southwestern LaPorte County, but have not seen the other 2 species. *C. tranquebarica* has been reported from Lake county (2). *C. limbalis* was found commonly by Shelford but has not been reported from Indiana, probably because its preferred habitat (steep clay banks) is not common here. Another species, *C. patruela* has been reported from several locations in northern Indiana (1, 2). Museum records also indicate this species was collected in several localities in northwestern Indiana.

Acknowledgments

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**Lesser Peachtree Borer:¹
Captures of Male Moths When One, Three, or Five
Females Were Used as Bait in Traps^{2 3}**

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Introduction

Since the female lesser peachtree borer, *Synanthedon pictipes* (Grote and Robinson), is highly attractive to the male (Girault 1907, Gossard and King 1918, Boyce 1962), the female moths have been used as bait in traps to monitor populations (Cleveland and Murdock 1964; Wong et al. 1969, 1971) and as a means of suppression of field populations (Wong et al. 1972). Also, the response of marked laboratory-reared males to increased numbers of bait females was evaluated (Wong and Cleveland 1972, Buriff et al. 1974) in release-recapture studies. However, little information has been obtained regarding the response of native male borers to various numbers of bait females in traps within a given trapping area. Therefore, a study was conducted to determine the relative attractiveness of 1, 3 or 5 females/trap in traps in peach orchards near Vincennes, Indiana.

Methods and Materials

Tests were conducted simultaneously during September and October 1973 in 5 commercial peach orchards. Test areas were blocks of trees (7- to 14-years old 'Redskin', 'Redhaven', and 'Cresthaven'), in rectangular plantings that ranged in size from 7 to 15 acres. The native populations of borers within the test areas were derived from existing trees heavily (35-50 larvae/tree) to moderately (15-30 larvae/tree) infested and from adjoining blocks of injured peach trees.

Bait females were laboratory-reared virgin females ranging in age from newly-emerged to 1 day old. These females were randomly collected from the emergence cage if they were in the "calling" position (Cleveland et. al. 1968) and transferred to holding cages for assignment to traps. Also, 1-2 small pieces of moist dental wicking were placed in the holding cages as a water source for the confined moths.

All traps were wing traps (Howell 1972) modified as described by Buriff and Davis (1974) (sold commercially as Pherotrap I®). However, the bottom of the

¹Lepidoptera: Aegeriidae.

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³Mention of a proprietary product in this paper does not constitute recommendation or endorsement by the USDA.

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traps had an additional coat of Stikem® to ensure the capture of most attracted males. Since all traps (3/block) were arranged in a straight line and were placed on the long side of each rectangular block, the 1st trapping station was centrally located ca. 60.97 m from the front edge of the short side of each test block; the distance from the 1st trapping station to the 2nd and 3rd stations was 121.95 m, respectively; and the final distance from the 3rd station to the opposite short side (back edge) of each block was ca. 60.97 m. The baits (1, 3 or 5 females) were completely randomized by female multiples and placed in traps hanging from branches 1.5 m above ground level. Also, as the traps were hung, they were positioned to allow free air movement and minimum shading, both of which influence female "calling" behavior.

Three times a week (9 AM-12 PM) for 35 days from September 3 through October 8, male moths captured in each trap were removed and counted. Also, all female moths were replaced and traps were repositioned in a clockwise movement. All trap bottoms were replaced weekly throughout the entire study.

Results and Discussion

A total of 4115 native male lesser peachtree borer moths was taken from the 15 traps. The percentage of the total trapped in blocks A, B, C, D, and E were 47.2, 15.1, 17.6, 8.8, and 11.3 (Fig. 1). This variability probably occurred because of differences between blocks in acreage, tree age, alternate hosts, and density of the borer population. Nevertheless, male attraction usually was directly related to the number of female moths per trap. For example, in blocks B, C, and E, catches of adult male moths were quite similar, and the response to increased numbers of bait females was definite. In blocks A and D, catches deviated slightly from the general trend during the 1st 2 weeks of the test period (September 3-17) but were more similar the last 3 weeks (September 18-October 8).

Peak field emergence occurred during the week of September 3, which produced high male activity (that present plus newly emerged) within and around all orchard blocks (test areas + infested adjoining areas). This increased activity was directly visible in higher catches per trap. Percentages of adult male captures from all areas (A-E) for the 1st 2 weeks of the initial (35-day) test period were 79.2, 80.6, 83.4, 84.2, and 79.1. The question therefore is whether the different numbers of bait females could really reflect their true potential in attracting males at a time of such density of the native population.

Field observations (personal communication, J. F. Howell, SEA, USDA, Yakima, WA) in extensive trapping studies of the response (male activity) of the male codling moth, *Laspeyresia pomonella* (L.), to pheromone emissions have tended to show that when attractive females are first introduced into a previously untrapped population, there is an excess of responsive males, and trap catches are higher. Then as the insect population is reduced to replacement status (rather than excess), the traps began to show their true attractancy. Apparently, in our study, conditions were such that there was an excess of responding lesser peachtree borer males during the 1st 2 weeks of the trapping periods. Data for the last 3 weeks then are more accurate and reflect better the response of the males to the different numbers of bait females (Table I).

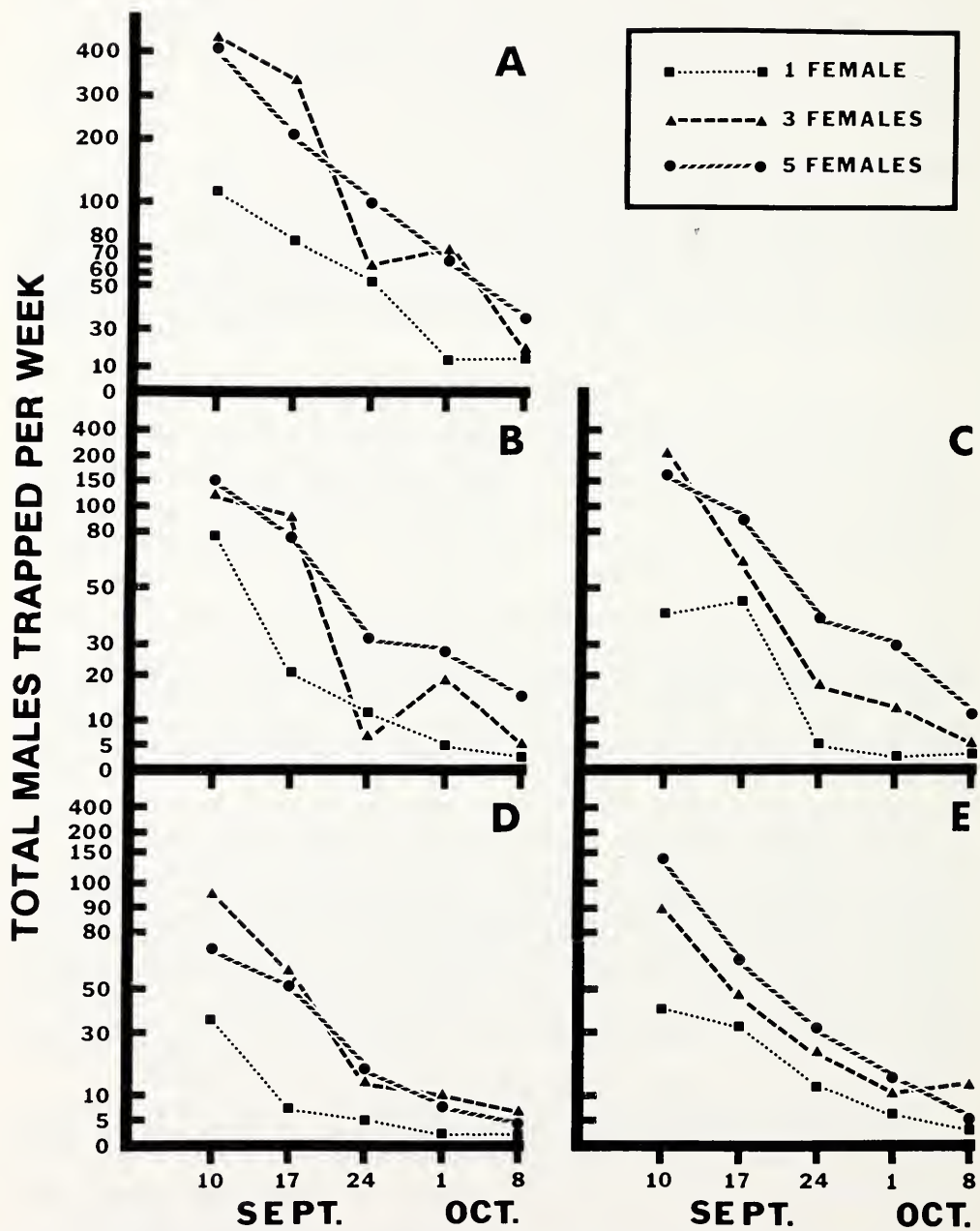


FIGURE 1. Comparative captures of male lesser peachtree borer moths in traps baited with 1, 3 or 5 virgin females in 5 commercial peach orchids, Vincennes, Indiana, 1973.

TABLE 1 *Captures of lesser peachtree borer males and attraction of females in orchard blocks (September 18-October 8), Vincennes, Indiana, 1973.*

Orchard	No. ♀/trap	Mean no. of ♂ trapped/week/trap		
		September	October	
		24	1	8
A	1	16.7	3.7	4.0
	3	19.3	22.0	4.0
	5	32.7	21.0	11.3
B	1	3.3	1.4	1.0
	3	2.0	6.7	2.7
	5	10.0	9.3	4.0
C	1	2.0	0.7	1.0
	3	6.0	4.7	1.0
	5	14.3	7.3	3.0
D	1	1.3	0.0	1.0
	3	3.7	3.0	2.0
	5	4.7	2.3	1.0
E	1	3.3	1.7	1.0
	3	7.0	2.7	2.3
	5	9.0	4.3	1.0

No definite explanations can be provided for the differences in male attraction in blocks A and D during the 1st 2 weeks of the test. However, there are several possibilities: (1) the initial placement of traps with regard to areas of localized infestation ("hot spots" in trapping areas), (2) increased male build up with heavy flight activity (male moths responding to closest pheromone source), (3) intermittent emission from the caged females (variable pheromone concentrations), and (4) trap orientation with regard to wind direction and air circulation (moth flight and orientation to pheromone concentrations). Any one or any combination of these factors would have influenced the results in these 2 test blocks, but all would apply equally to male activity in these blocks and other orchard test areas.

A distance of 121.95 m between trapping stations was selected in hopes of preventing interaction between the females confined in traps within the same orchard. Interaction apparently was prevented since 1 female/trap caught 23.6 male/female (female equivalents based on total catch from all test areas) and 3 and 5 females/trap caught 22.7 and 14.6 male/female respectively. Usually trap catch was somewhat proportionate to the number of females per trap.

(Fig. 1) illustrates the principal difference between using 3 or 5 female multiples per trap, not only was the number of males caught increased but the reliability or consistency of the catch was improved. This probably relates to pheromone emission. Where there were only 3 females there were probably more interruptions in the emission pattern than with the 5 female multiple. We can conclude that for both survey and control purposes, traps baited with 5 females would be more efficient and reliable than traps baited with 3 females.

However, in the area of male response investigation, the optimum number of females per trap is not established. Neither is the number and placement of traps needed to regulate or suppress native populations of this insect.

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Physiological Characterization of the Migratory Milkweed Bug, *Oncopeltus fasciatus*

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Introduction

Oncopeltus fasciatus is a migrant colonizer invading northern areas such as Saint Joseph County, Indiana each spring from more southern areas to establish one or more generations during the summer. The population does not overwinter. Some individuals may migrate south in the fall but most are killed by harsh winter conditions (1, 3).

Laboratory cultures of *Oncopeltus* grown at 23°C with a 16 hour photoperiod exhibit long duration tethered flight during the post-teneral prereproductive period. When a 30 minute flight was used to determine migratory organisms, 30% of females and 18% of males of a wild type population were migratory. When these individuals were inbred, the percentage of migratory organisms was increased in the offspring to 60-70% thus demonstrating the genetic basis of this behavior (3).

The ability to fly involves the presence of wings, flight muscles, mitochondria with the ability to produce sufficient ATP and the ability of the fat body to accumulate and release metabolites. A lesion in any part of this chain would lead to a reduced ability to fly. The purpose of this study was to demonstrate that there were several biochemical differences distinguishing the migratory strain from the rest of the population.

Materials and Methods

Rearing: From a wild type laboratory culture of *O. fasciatus* derived from individuals originally collected in Saint Joseph County, Indiana in 1973, a migratory strain and a sedentary strain were selected. Migratory organisms exhibited 30 minute tethered flight during the post-teneral prereproductive period. Sedentary organisms did not. The strains were inbred and annually subjected to additional selection. Colonies were reared in gallon glass jars on milkweed seeds (*Asclepias syriaca*) and water at $23 \pm 3^\circ\text{C}$. Wads of cotton were provided for oviposition sites.

Protein determinations: Protein concentrations of eggs, third instar nymphs, adult males and adult females were determined by the method of Lowry *et al.* (11) after masceration in a Ten Borek tissue grinder in 0.5 N NaOH. Eggs were collected over a 24 hour period for extraction in groups containing between 20 and 85 eggs weighing between 6.5 and 35 mg respectively. Eggs of the migratory strain were derived from pairs of insects each of which had flown for 30 minutes prior to mating and egg laying. Third instar nymphs and adults of both sexes were extracted individually.

Lipid determinations: Lipids were extracted from groups of adult *O. fasciatus* of each strain with a wet weight of between 1 and 5 grams by the method of Folch *et al.* (5). Total lipid samples were evaporated to dryness over steam and under a partial vacuum in a preweighted flask. The flask was then reweighed and the difference recorded as the lipid present in each sample. Lipid content was expressed as the grams of lipid per gram live weight.

Mitochondrial isolation and enzymatic activities: Four thoraces were pooled and homogenized in 2 ml of cold 250 mM sucrose plus 2 mM EDTA (pH 7.4) isolation media. The homogenate was filtered through double layered cheesecloth and the mitochondria were prepared by differential centrifugation using a rapid isolation technique (10). Cytochrome c oxidase activity was measured on a Beckman Dual Beam (DBG) recording spectrophotometer as the rate of decrease in absorbance of reduced cytochrome c at 550nm in a previously described reaction mixture (9). Specific activities were based on the protein content of the mitochondrial samples as determined by the Folin-phenol method of Lowry *et al.* (11).

Results

(Fig. 1) shows the mgs of protein per mg wet weight for eggs, third instar nymphs, adult males and adult females of wild type, migratory and sedentary strains of *O. fasciatus*. No significant differences in protein content were demonstrated in eggs. Third instar nymphs of the migratory strain contained significantly less protein than wild type or sedentary strains. Adult females of the migratory and sedentary strains contained significantly more protein than the wild type strain. Adult males of the migratory strain contained significantly more protein than wild type or sedentary strains.

Table I compares the percent lipid of the wild type, migratory, and sedentary strains of *O. fasciatus*. The wild type strain contained 12.1% lipid; the migratory strain contained 25.4% lipid; the sedentary strain contained 5.4% lipid.

TABLE I *Lipid Composition of Wild Type, Migratory, and Sedentary Adult Oncopeltus fasciatus*

Strain	% Lipid
Wild Type	12.1 (3)
Migratory	25.4 (3)
Sedentary	5.4 (3)

The numbers in parenthesis indicate the number of replications. Each value differs significantly ($P < 0.01$) from the others.

(Fig. 2) shows the activity of cytochrome c oxidase from the flight muscle mitochondria of wild type, migratory and sedentary *O. fasciatus*. Cytochrome c oxidase activity differs significantly and is three fold greater in the migratory strain than in the sedentary or wild type strains.

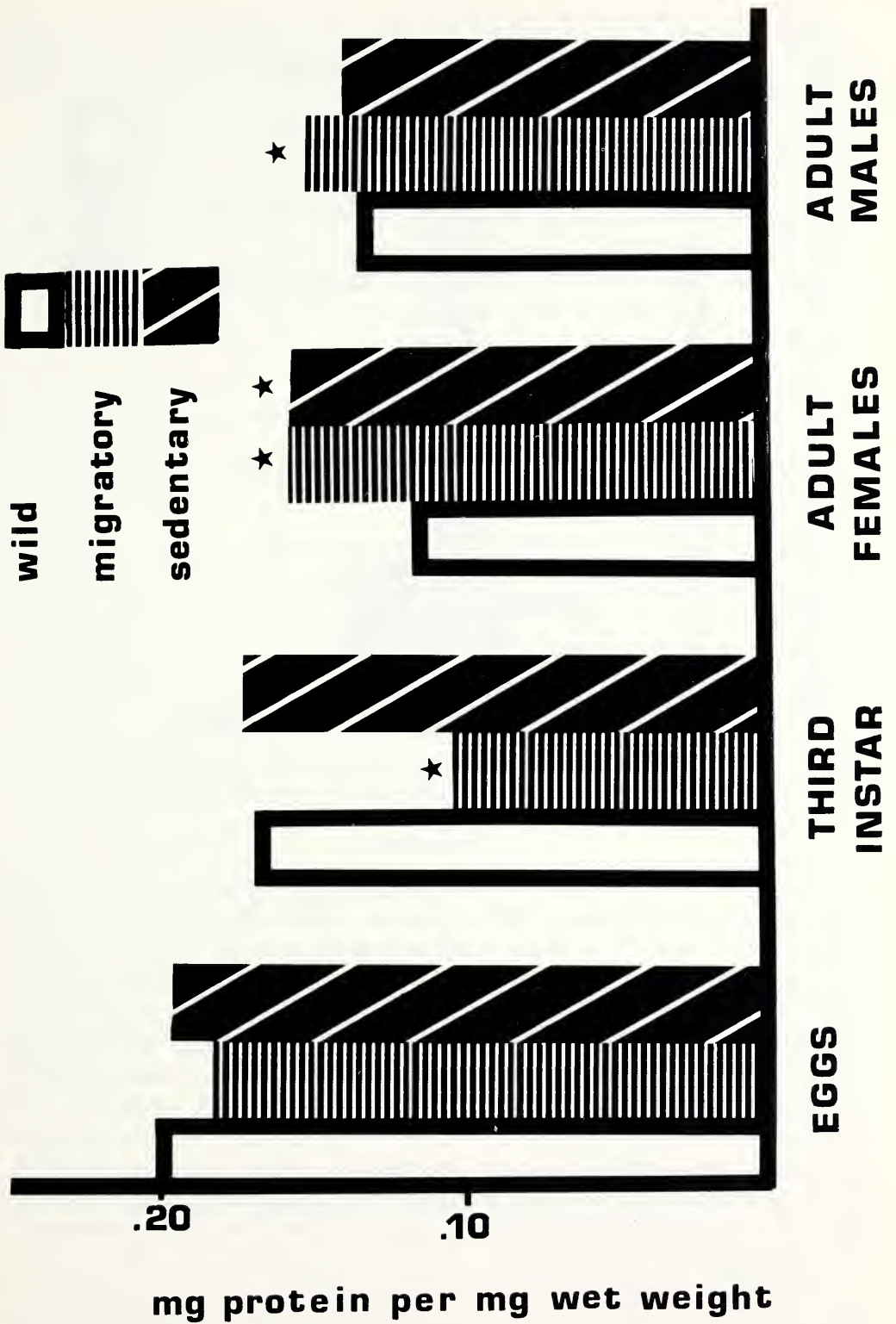


FIGURE 1. Protein concentration of wild type, migratory, and sedentary *Oncopeltus fasciatus* during egg, third instar and adult stages. Each bar represents the mean of 12 determinations. ★ represents statistical significance.

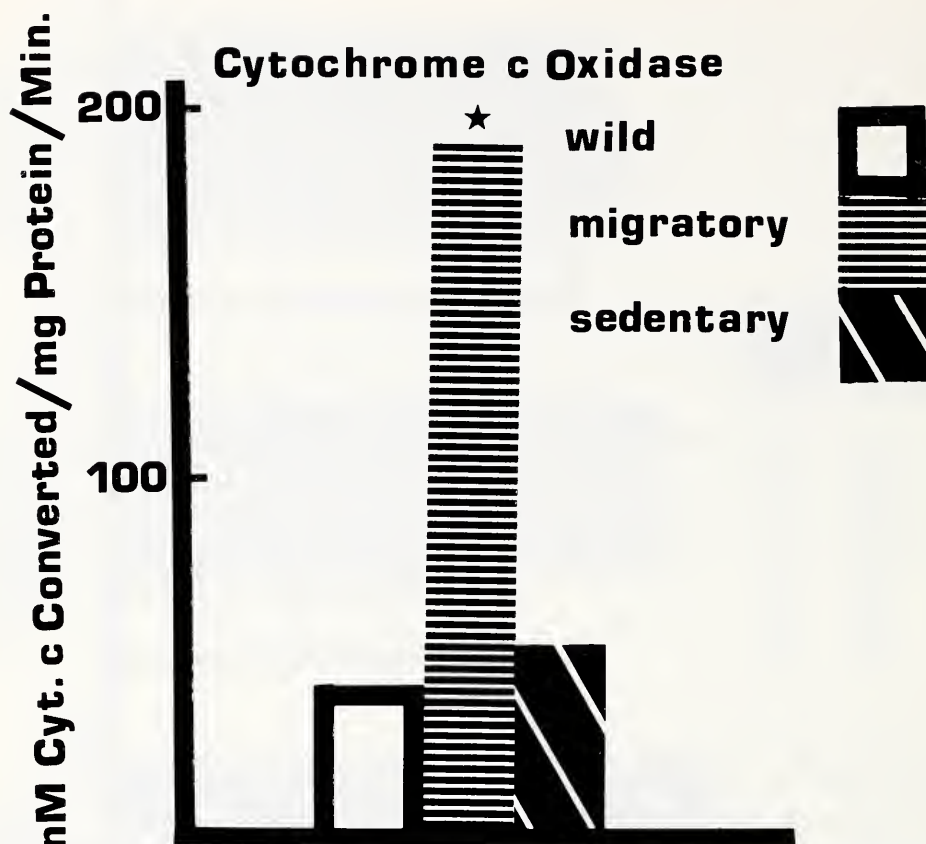


FIGURE 2. Cytochrome c oxidase activity in wild type, migratory and sedentary *Oncopeltus fasciatus*. Each bar represents the mean of 4 determinations. ★ represents statistical significance.

Discussion

The results of this study indicate significant differences in total protein, total lipid and cytochrome c oxidase activities between wild type, migratory and sedentary strains of *O. fasciatus*. While large numbers of studies have followed protein and lipid composition of insects at different developmental stages (15), relatively few have compared flight forms with flightless stages. The protein concentration of prenuptial flight *Solenopsis invicta* decreased 50% during the first 12 hours after flight while lipid concentration remained unchanged (16). The flight form of the adult cowpea weevil, *Callosobruchus maculatus*, had nearly twice as much total body lipid as did the normal weevil (13). These authors did not investigate protein concentration. While earlier studies of *O. fasciatus* characterized the fatty acids of different mutants (6) no studies of the total lipids of migratory and sedentary forms of the milkweed bug are known to these authors.

The studies of flight muscle mitochondrial enzymes can be grouped into three categories: 1, developmental or aging studies in which all the members of the population are considered fliers but not necessarily migrators (7, 8); 2, those comparing a species of normal fliers with another species which is flightless (12); and 3, those describing a totally migratory species (2). Such information must be used carefully when comparing those enzymatic activities to these for migratory strains of *O. fasciatus*. The activity of cytochrome c oxidase reported here for the

migratory *O. fasciatus* is the same as that reported for *Heliothis virescens* (8). The general idea that the enzymes of flight muscle mitochondria of fliers differs from non-flying forms is substantiated in this study.

Juvenile hormone has been shown to mediate migratory behavior in *O. fasciatus* primarily through changes in esterase activities destroying the hormone (14). It is unclear at this time how the hormonal changes influence lipid or protein composition and the enzymes of the flight muscle mitochondria. Since there appear to be so many biochemical differences separating the migratory form of *O. fasciatus* from the rest of the population, it is probable that no one simple explanation will be found to explain migration.

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**Associates of the native bee, *Halictus (Seladonia) confusus* Smith
(Hymenoptera: Halictidae)**

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Introduction

The ecological life history of a local population of *Halictus (Seladonia) confusus* Smith, a common halictine bee of the eastern United States, was studied near West Lafayette, Indiana, from 1963 to 1965 (4). This species is a ground-nesting bee that nests in aggregations, primarily in open fields, and prefers bare or sparsely vegetated sites. In central Indiana, the species is primitively social: Nests are begun by solitary females and have the potential to develop into matriarchal associations with a mother queen and daughter workers. The workers enlarge the nest and construct and provision cells in which the immature stages develop. Two to three broods are produced each summer.

The female castes are morphologically inseparable, and range from 6 to 8 mm long. Whitish fasciae on the apical margins of the tergites of this dark metallic green bee give the abdomen a banded appearance. Adult females, including inseminated insects that overwinter in parent nests, are present throughout the year. Males are present in all broods but do not overwinter. The species is polylectic, and adults may be found frequenting many field and woodland flowers from mid-March to late September.

Several predators and parasites were noted attacking larvae and adults of *H. confusus* during 3 summers of observations of a nesting site. Because mortality of the bee species seemed more the result of predators and parasites than other causes, special attention was given to these biological agents and their interrelationship with *H. confusus*.

One advantage to the bee species of a social nest rather than a solitary nest is the presence of a guard at the entrance to repel predators and parasites (6). Another advantage of the social nest is that the queen, which stays in the nest, is less subject to the attacks of above-ground predators and parasites. In the nest, a defending bee is able to cope with an intruder effectively since the bee body nearly fills the nest entrance and the adjoining horizontal tunnel, and the bee mandibles serve as formidable defensive weapons. The females can also brace themselves tightly against the tunnel walls with the basitibial plates and, in this position, cannot be easily dislodged nor bypassed by intruders. In contrast, during the spring solitary phase, predators, parasites, and alien bees often entered an open nest during the absence of the foraging bee. Such invasions subsequently became uncommon due to the presence of a nest guard once a worker caste was produced.

Predators.—A mummified male *H. confusus* found in a flower suggested that some bees fall victim to crab spiders and ambush bugs common on field

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flowers. However, ants were the major predators of the nesting area, and 4 species were observed attacking bees resting in the nesting area or the nest-guard bee. Ants also entered open, unguarded nests, but most ant attacks could be avoided by flight of the bee that was outside the nest or by aggressive action of an adult bee that was within the nest. One attack was witnessed in which a cluster of about 10 *Aphaenogaster rudis rudis* Emery engaged the guard bee at a nest entrance in direct attack. Several of the ants enlarged the entrance by removing particles of soil which were transferred from one ant to another and passed to the outside. Time passed, and more ants arrived and continued to remove soil. Eventually, the ants overwhelmed the guard and thereafter entered and left the nest at will. Several ants left the nest dragging the mutilated guard now stripped of her wings and with 1/3 of her abdomen removed. Another ant carried a white pupa of *H. confusus* in its mandibles. Until dusk, the ants entered and left the nest periodically. The next day, the ants were gone, and, suprisingly, a female *H. confusus* without a pollen load entered the nest. The nest then remained closed for 11 days and then reopened attended by a guard. Two days later, provisioning workers were present, and the nest remained active for the remainder of the summer. Lone individuals of this ant species have been observed attacking a nest guard in other nests, but these attacks were always successfully repulsed.

Lasius alineus (Foerster) was another common invader of the *H. confusus* nests. This ant frequently entered nest entrances but retreated quickly when contact was made with the guard. Also, in one instance, an ant of this species started to enter an unguarded nest but then retreated rapidly as if a repellent secretion was deposited around the inside of the entrance by the bee that constructed the nest. This ant species was in newly-abandoned *H. confusus* nests. It quickly located the closed cells and removed both the pollen provisions and the immature stages by cutting off and removing pieces of these stages until no trace remained. Open abandoned nests were quite vulnerable to this species, and ants were observed entering and departing from such nests as long as salvageable nutrients remained. However, nests with no signs of bee occupancy other than deserted burrows and empty cells often had these ants present in the tunnels. In these cases, ant tunnels intersected with the nest burrows and the ants had free access to the bee nest from their own nest.

Thief ants, *Solenopsis molesta* (Say), were too small to pose a threat to a defending female *H. confusus* and were never observed attacking en masse. However, they were common and joined with *L. alienus* in attacking the immature stages in the cells during a nest dissection if a nest undergoing dissection was left untended for several hours. In natural situations, the thief ants normally were not found in the main tunnels of the nest or contacting adult bees. However, on occasion, they entered cells from tunnels that penetrated the cell walls and cleaned out the contents of the cells.

Formica pallidefulva nitidiventris Emery workers were conspicuous in the nest area due to their large size. The ants were never observed in the nests and did not probe the nest entrance, as did other species. The only time they were attracted to the entrance was when a grass straw was inserted in the entrance by the author and left there for a period of several minutes or more. Even under artificial conditions, the ants did not attempt entry or engage the guard. The

only observed physical contact was with inactive adult bees in the nest area. Several times an ant was seen approaching and touching a resting female bee, which responded by flying a short distance. Ants carrying moribund female bees in their mandibles have been observed, but it is not known if the bees were functional adults captured by the ant or bees that were spent or parasitized and nearing death before capture.

Paratrechina parvula (Mayr) ants were casual probers of nest entrances but always left when repulsed by the guard. They were never found in the nests.

All ant species were determined by D. R. Smith (AR, SEA, USDA).

Adult parasites.—Dipteran parasites of the family Conopidae were the major parasites of adult female *H. confusus*. It was not uncommon to find female bees that had died and were in various stages of decomposition within the nests. If the responsible parasite had emerged, the abdomen of these bees was likely to contain a puparium or its remains, which completely filled the abdominal space. If decomposition was advanced, the abdominal sclerites separated, and the puparium was evident without dissection of the bee. Dead bees containing live conopid puparia have been found at the base of the nest in digging position, embedded in the sides of the nest, and in old cells. If the nests were active, the dead bees were walled off or their bodies were apparently bypassed in further excavation of the nest, depending on the position of the body in the nest. One cell containing a dead bee was filled in with soil by active workers.

Larvae of 2 species of Conopidae have been recovered from the haemocoel of the abdomens of active female bees; in addition, an adult fly reared from a puparium has been identified as *Thecophora occidentalis* (Walker) by G. Steyskal (AR, SEA, USDA). Both of the larvae differed from *Occemyia* reported by Kirkton (5) as a parasite of the bee *Halictus ligatus* (Say), which was found in the same locality. The first dead parasitized bee was found on May 10, 1964, at the base of a new nest under construction at the time of death. In nest dissections during midsummer 1965, several marked bees of the spring solitary phase that would later become queens and also some first-brood workers were parasitized by conopids. Only 1 parasite per bee was found. The conopid larvae did not mature and pupate until after the death of the host.

Adult conopid flies were usually present in the nest area during favorable weather. They rested on the vegetation and struck at passing female bees in flight. Quite often the fly and bee tumbled to the ground during these brief encounters. Bees returning to the nest were often attacked, but bees leaving the nest were not approached. During the nesting seasons of 1964 and 1965, 9 of 58 nests dissected were found to contain live or dead females that had been parasitized by conopids.

Phalacrotophora halictorum (Melander and Brues) (Diptera: Phoridae) (det. By W. W. Wirth, AR, SEA, USDA) were frequently observed near the entrances of *H. confusus* nests. On a number of occasions, a small phorid adult was observed attempting to enter a nest, only to be repulsed by the guard. Eventually, the fly assumed an immobile stance near the nest entrance; then when a returning worker entered the nest the parasite followed close behind. It

would remain in the nest for several minutes. Similar behavior by this dipteran species has been reported by Melander and Brues (8) for nests of the bee, *Lasioglossum pruinosum* (Robertson) and by Batra (2) for *L. zephyrum* (Smith).

On two occasions, small nondescript maggots were recovered from the abdomen of healthy females. These were determined to be parasitic Phoridae by G. Steyskal (AR, SEA, USDA). In one instance, 2 maggots were recovered from the abdomen of 1 female.

One nondescript nematode was found in the abdomen of a healthy larva on July 18, 1964. It was determined by J. M. Ferris (Entomology Department, Purdue University). Gregarine sporozoans (Eugregarina, cephalina) were often present in the intestine of the bee anterior to the rectum. These unicellular bodies were translucent, oval, and partitioned unequally into 2 regions. They measured about 0.1 mm long and were visible at 15X magnification.

Larval parasites.—The parasitic beetle, *Rhipiphorus walshi* LeConte (det. T. J. Spilman, AR, SEA, USDA) was the primary parasite of the larvae of *H. confusus*. The adults were common in the nesting area, and their immature stages were present in many cells of active nests. This species of parasite has a complex life history: Adult female beetles lay eggs on the blossoms of field flowers. The eggs produce active, 2 mm, first-instar larvae (or triungulins). When a female bee visits the flowers, the triungulins use their mandibles to attach themselves tightly to parts of the bee. From 1 to 4 triungulins have been found attached to 1 bee, most of them in the wings near their base. A few have been found clinging to the hair of the legs and abdominal sternites.

Triungulins were most common on bees during the spring solitary phase (immobile and inactive state) prior to the construction of cells. They were then in a phoretic association with the hibernating females for 8 to 9 months, from August or September until the following April or May. In spring, as the solitary females constructed the first pollen balls of the year, the triungulins left the bee and remained in the sealed cell with the pollen ball and egg. Several active triungulins were observed on a fresh pollen ball before the egg had hatched. As the egg hatched and the bee larva began to grow, the triungulins burrowed into its abdomen. There it remained relatively inactive and did not attain much growth as the bee larva developed into a prepupa. However, after a molt, it burrowed to the outside and attached itself at a point just behind the head with its body curled around the thoracic segment of the host. One parasite per host was the usual number though 2 were observed in one instance. Parasitized bee larvae never matured beyond the prepupal stage and all died, whether the parasite larvae survived or died. As an ectoparasite, the larva fed continuously, with a conspicuous bellow-like action of its head and thoracic region; so in 3 to 5 days, the host was reduced to a small bladder-like body of liquid. By 6 to 7 days, the parasite was fully grown, and the host was reduced to a dried mass of exoskeleton. The fully-grown parasite was comparable in size to a bee prepupa. During the feeding process, the parasite was not observed molting, nor were any cast skins found.

The fully-grown parasite was quiescent for 1 to 2 days and then molted to a white pupa. Sex of the pupae could be determined since antennae of the female

are shorter and narrower than those of the male. One day after pupation, the eyes darkened to a tan color; by the 2nd or 3rd day they darkened further. On the 4th or 5th day, the pupa began to darken. Until it emerged at 6 to 8 days, the abdominal segments had alternating dark and light bands. Within a short time after emergence, the newly-emerged adult discharged a large amount of a white liquid from its mouth onto the cell floor or rearing chamber. The adult beetle remained in the cell for 1 to 2 days while it dried and became better able to walk about; its abdomen darkened further and contracted somewhat until most unsclerotized regions of the abdomen were telescoped internally. The adult beetles then pushed out the soil plugs of the cell and emerged as free-living adults. A newly-emerged female was strongly attractive to males of the same species, possibly because of a sex pheromone: when a female beetle was captured and held in a net for several minutes, as many as 17 males were attracted to the female, and collected. These males had a remarkable range of size, from 2.6 to 5.7 mm.

After mating and egg maturation, the female beetle oviposited upon field flowers to complete the cycle. Then triungulins were again transported from the flowers to cells. However, the association with a worker at this time could be as short as a few minutes, unlike the long fall to spring association. For this reason, in the summer triungulins were not found as often attached to bees as in spring before the cells were constructed.

There appeared to be 2 to 3 generations of beetles each year. In a density-dependent mechanism, the presence of this parasite became more common as the season progressed. The highest percentage of parasitism recorded in a nest was 50% in late summer. The extent of parasitism could not be accurately determined since many immature larvae died during laboratory rearing or were preserved without checking for endoparasites. Freedom from parasitization could not be assumed until the bee entered the pupal stage.

Rhipiphorid parasitism was apparently the major population-limiting factor for the *H. confusus* in the study area. A similar host-parasite relationship for an undetermined *Phipiphorus* was reported by Barber (1) upon *Augochlora pura* (Say) and by Linsley, MacSwain, and Smith (7) for *R. smithii*, upon a parasite of *Diadasia consociata* Timberlake. Batra (2) reports the occurrence of *Rhipiphorus fasciatus* (Say) in the nests of *L. zephyrum*.

Other Associates.—An anoetid mite species of the genus *Histiostoma* (det. by E. W. Baker, AR, SEA, USDA) had an obligatory commensal relationship with *H. confusus* and did not noticeably interfere with the various life stages. These mites were oval and reddish-purple in color. They fed upon the pollen balls in the cells at the same time as the bee larvae were feeding and growing. As the adult bee emerged, the mites attached themselves to the body of the bee, sometimes the wings, but usually to the first abdominal tergite, and became inactive. A latticed aggregation of 85 mites was counted on one such mass of the abdomen of an overwintering female. The mites eventually left the host female bee to feed upon the pollen ball of another cell in completion of the life cycle. These mites were in phoretic association on workers for brief periods in the summer, and the association on the overwintering females lasted for periods of 8

to 9 months. The mites were common on spring females before cells were constructed but were uncommon on summer workers.

A number of irregular visitors, saprophytic organisms and scavengers were observed on *H. confusus* from time to time. Particularly, the overwintering nest served as a refuge for species other than *H. confusus*. A small spider and a 6 mm *Dialictus* sp. female were noted in nest dissections.

Moldy pollen balls supported numerous scavengers such as nematodes and spherical-bodied mites.

A tiphiid, *Myrmosula parvula* (Fox) (det. by K. V. Krombein, Department of Entomology, Smithsonian Institute) was captured in 1 nest burrow during a nest dissection, and several others entered unguarded spring nests. No parasitic association was demonstrated, however, and the parasite may have been searching for another bee species.

Dialictus imitatum (Smith) females sometimes entered *H. confusus* nests during the absence of the female in the spring solitary period. However, they never remained in the nests for more than 30 minutes and left when or before the owner returned. Occasionally, male *Calliopsis andreniformis* Smith struck at *H. confusus* females in flight but made no attempts to follow the females into the nests.

One dead and soil-covered female *H. confusus* was found packed full of spherical bodies filled with moisture. These bodies may have been some species of fungus.

During rainy periods, excessive moisture accumulated in some cells and a fungus would become established on the pollen ball and grow into a mass of white hyphae. Eventually, all of the pollen was destroyed, and as the fungus grew, the larvae died. In time, the fungus attacked the body of the larva also. Chandler (3) reported *Gibellula pulchra* in the nests of *H. ligatus* by the fungus. In the present case, many of the cells bearing fungal deposits had been filled with loose soil, which indicated that the fungus had been detected by the nest bees and blocked off in the same manner that dead parasitized adults are sealed off with soil. Since fungus grew on fecal deposits in old cells, which had served to produce the brood, the practice of the worker bees of filling these cells with loose soil was probably related. In all of these cases, the end result was the isolation of fungus growths from the rest of the nest.

Acknowledgments

Thanks are extended to Dr. R. L. Giese, formerly of the Department of Entomology, Purdue University, for taking the photographs shown in the article and to Dr. Leland Chandler, my major professor, who provided invaluable guidance. The determinations made by various taxonomic specialists were especially helpful.

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GEOLOGY AND GEOGRAPHY

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Abstracts

Pontoon Boats: An Indiana Recreational Industry. THOMAS P. GRIMES, Ball State University, Muncie, Indiana 47306._____Pontoon boats, popular recreation craft on sheltered inland lakes in the United States, have been built in Indiana for over a quarter of a century. More firms manufacturing pontoon boats are located in Indiana than in any other state. Investigation suggests that current pontoon boat fabricators were in business manufacturing farming equipment or subassemblies for farming equipment before adopting pontoon boats as a product. This paper will discuss the pontoon boat manufacturing industry in Indiana in terms of its history, location, local impact, and national output. Pontoon boats will also be considered within the context of other waterborne recreational vehicles as to their effect on the environment and as to their requirements for water space.

Soil Assessment Basis for Tax Determination in Delaware County. BENJAMIN MOULTON, Indiana State University, Terre Haute, Indiana 47809._____The celebrated reassessment of Indiana mandated to be completed a year ago and now delayed until this year with the tax to be paid in 1980 caused considerable difficulties for local assessors. In Delaware County the difficulties resulted in court action to determine the direction and personnel to carry out the evaluation. The procedures and results are the substance of this paper. The resources available to the researchers consisted of first, a new type soil survey, and secondly a new style tax parcel map on a scale of 1":400' for most of the county with a few maps on a scale of 1"-100'. The research workers identified farms by number only and were known to the map worker by number only. The instrument used for calculation of acreages was a Numonic #1224 digitizer. The acreage of each soil type on each farm was measured. The assessed value was analyzed further with production ratings of soil types determined by the staff at Purdue University and a computer program designed to determine the assessed value of a farm with allowances being made for home sites and areas of non-production (drain ditches etc). The bottom line of the computer program is the total assessed value for each farm, each township, and finally a county total of all farms and farm land.

An Ecosystem Inventory of the Indiana Dunes National Lakeshore. WAYNE E. KIEFER, Central Michigan University; CARL KREKELER, Valparaiso University; GAYTON MARKS, Valparaiso University; MARK RESHKIN, Indiana University-Northwest; NEIL WEBER, Indiana University-South Bend._____An ecosystem inventory has been compiled for the Indiana Dunes National Lakeshore. The

inventory covers the following: climatology, flora and fauna, geology, land use, and soils. The geology, land use, and soils have been mapped at a scale of one inch to four hundred feet. Climatological information has been presented in statistical and cartographic form. Lists have been prepared of the flora and fauna found within the Lakeshore boundaries. The inventories, along with supporting text, have been prepared for the National Park Service and are intended to provide a base line from which ecological change can be measured as well as a data base from which ecologically sound management plans can be developed.

The Flood Hazards of the St. Joseph River in North Central Indiana. KENNETH R. BREHOB, Department of Earth Sciences, University of Notre Dame, Notre Dame, Indiana 46556. _____ Flood prone areas along the St. Joseph River within Elkhart and St. Joseph Counties were identified and residents of these areas were interviewed to determine their flood hazard perception. Five flood prone residential areas were selected for questionnaire interviews using a slightly modified version of the standard natural hazard questionnaire. Selected interview results are presented. Lack of awareness of a flood hazard was typical of the 78 questionnaire respondents. Possible reasons for the low perception level of the flood hazard includes: lack of major floods since 1950, high residential mobility, and confidence that "river flow" dams control water levels during flood events.

An Alternative Hypothesis for the Natural Formation of Opal

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Introduction

The structure of opal was first elucidated by P. J. Darragh and A. J. Gaskin in 1966, (1), working in two laboratories of the Commonwealth Scientific and Industrial Research Organization, in Perth and Melbourne, Australia. In their first paper they also suggested a natural origin of the structure. A recent paper, (2), summarized the findings of continuing work from those laboratories to 1976.

These authors determined, largely by electron microscopy, that opal possesses a structure which resembles a system of packed spheres. In some opal the size of the spheres is very uniform and the packing is that of a regular lattice, either hexagonal close packed or face centered cubic. In others the size, shape and packing are very irregular. No compositional distinction has been reported between the two.

“Precious opal” is so called because it possesses a “play of color” which was clearly recognized as an optical diffraction phenomenon and was shown by Darragh and Gaskin to originate from the regular lattice structure. “Common opal” has no regular lattice structure and possesses no “play of color”. Precious opal, whether formed in sedimentary or volcanic rocks was found to have the regular lattice structure, though many features of the two such as amount of crystallinity and the appearance of electron micrographs of cleaved surfaces were quite different.

These authors proposed an hypothesis for the natural formation of opal (1, 2) which may be referred to as the “sedimentation hypothesis”. Their hypothesis postulates the formation and sedimentation of spheres of silica in a pool of a very dilute but saturated solution of silica. The solubility of silica at pH below 9.0 is approximately 0.015%, (3). Such pools were said to be in a steady state in which supply by entry of new silica solution is in balance with evaporation of water from the pool, precipitation of silica spheres from the saturated solution, and aggregation and settling of aggregated spheres into a regular lattice of uniform spheres as revealed in their electron micrographs of precious opal.

After this process of settling of silica spheres into a close packed lattice is complete, calculated by those authors to take from one to several millions of years, dehydration of the mass is postulated to convert it to the opal state.

Those authors were able to form silica spheres by hydrolysis of silica esters and to sediment them into a regular array which was said to display play of color. They were not able to consolidate the mass into an opal-like solid. A U.S. patent was obtained by the authors, (4).

To date no other hypothesis has been put forward for the natural formation of opal, to explain the well documented lattice structure of precious opal. A secret process has been found and used by Pierre Gilson of Paris, France by which a stone is produced having optical effects closely resembling natural opal and said to have the same lattice structure seen in natural opal, (5). This secret process can have little relationship to the process of natural formation as postulated by Darragh and Gaskin, (1), which requires one to several million years.

An Alternative Hypothesis

In sharp contrast to the sedimentation hypothesis it is here proposed that a process is involved which begins with the entry of a magmatic alkaline solution of silica into a cavity in a host rock or alternatively into porous masses such as dried wood or porous earth. Precious as well as common opal can form in either situation. At pH 11.0 the solubility of silica is 0.45% and would approximate 4.0% at pH 12.0.

An alkaline solution of silica intruding upward should remain fluid until it reaches levels close enough to earth surface to come under the influence of carbon dioxide and oxygen from the atmosphere. If a pool of alkaline silica solution is formed close to earth surface it should come under the acidifying effects of carbon dioxide and of acids formed by oxidative processes. The pool should then become acidified. If the solution contains 4.0% or more of silica it should gel upon acidification, as is readily observed in the laboratory. Even lower concentrations may gel upon acidification and long standing. Also evaporation from a more dilute alkaline fluid solution may concentrate it to a degree allowing it to gel upon acidification. Note that in the sedimentation hypothesis the formation of a gel is not postulated to occur.

Intrusion of alkaline (high concentration) silica solution into the host cavity may occur in successive surges, causing the appearance of layers—sometimes thick, sometimes very thin—in most opal found in sedimentary host rocks.

The successive layers are formed of silica solution differing in such characteristics as concentration of silica, pH, presence of other dissolved constituents, notably carbonate and di- or multi-valent metal ions, etc. Also between the surges of intruding silica solution some evaporation and some acidification and gelling of earlier layers may occur. This may account for absence of mixing of new layers with older layers and the generally sharp and flat layering of sedimentary opal. In opal formed in cavities in volcanic rocks such layering is minimal or absent.

After intrusion of alkaline silica solution into host cavities and after acidification and gelling of the pool, the gel may remain relatively stable for many years before it begins to be dehydrated, or it may begin to dehydrate soon after gelling. It is generally believed that most opal forms under arid surface conditions. It is here proposed that during ageing and dehydration of the gel a structural rearrangement of the hydrated silica occurs in the gel mass. The rearrangement may be supposed to increase the number of Si-O-Si bonds at the expense of Si-O-H bonds.

When silica gel is dried it shrinks very little—unlike many other gels. The implication is that a structure remains in the dry gel which is ninety percent void space, (from a five percent gel retaining an equal weight of water). The present hypothesis suggests that under appropriate conditions, some of which will be suggested, the retraction of silica from its fully hydrated sponge structure in the gel may form a regular honeycomb or closed cell foam structure. These cells must be filled with gas as water is lost. The gas is predominantly carbon dioxide with some oxygen and water vapor. Throughout this process surface tension must play an important role, the more so that the dimensions of the cells are so small.

A mechanism is suggested in the following by which the cellular foam may become established during the course of drying of a silica gel. When soluble silica is acidified and gelled it was observed by conductivity measurements to undergo little conductivity change, indicating that the gel has a sponge structure rather than a closed cell structure. Upon dehydration of the gel energetic considerations would require some kind of further polymerization of silicic acid.

If gas cells are nucleated in the gel as it begins to dehydrate perhaps by carbon dioxide such gas bubbles could not move in the gel medium. As the water leaves, however, the bubbles could grow until they become close packed with the hydrated silicic acid forming the septa between and occupying only some ten percent of the gross volume of the original—and also of the dried gel. Such mechanism of retraction from the sponge—to a well polymerized form of hydrous silica would appear energetically plausible.

At this stage also the now dry silica structure is stable until a later intrusion of silica bearing solution occurs. The closed cell foam may be a regular lattice of uniform sized cells or it may be irregular with respect to cell size, shape, and arrangement. The obvious suggestion then is that this is a precursor of the later development of the opal structure.

To become opal this foam must be silicified. In increasing degrees and perhaps in successive stages it may become silicified by deposition of chalcedony, opal-cristobalite, or hydrous silica. The process is supposed to be similar to or identical to that by which other porous bodies are silicified.

The widely observed banding of the spheres in precious opal, visible in the electron micrographs in reference (2) may be due to successive phases of silicification rather than to the mechanism suggested in the sedimentation hypothesis. The present hypothesis supposes that silicification builds upon the inner surfaces of the cells of the silica foam. The structure remains permeable to a true solution of silicate, and undergoes a series of filling—gelling and drying with retraction onto the previous surfaces. This would result in concentric rings of silica within the cells of the foam. Some opal shows some degree of crystallinity (opal-cristobalite) while some is purely amorphous.

While opal formed in cavities in sedimentary rock typically shows layering, that formed in cavities in volcanic rocks generally does not. Also, while layering of the spheres in sedimentary opal suggests successive surges of silica solution during filling of the foam no layering in the spheres of volcanic opal has been reported. This suggests that fewer surges of silica solution are involved in formation of volcanic opal.

The well known variation in friability of opal and resistance to the stress of drying or heating on its stability is believed to be largely attributable to the varying completeness of the silicification stage in the natural development of opal.

An important aspect of this foam hypothesis is the proposed role of carbon dioxide in the formation of the foam on ageing/drying of the gel. It is proposed that the alkaline silica solution from which the pool is formed contains dissolved carbonate in an amount at least about 0.4 gram (calculated as sodium bicarbonate) per 100 ml solution. This is an amount which upon acidification produces enough carbon dioxide to saturate the solution at 25° C, and also the amount of carbon dioxide necessary to fill the voids left by evaporation of water from the gel. Accordingly carbonate content of the alkaline silica solution may be an important variable in opal formation.

The Nature of Evidence for the Foam Hypothesis

(1) A precedent appears to exist in the structure of novaculite, which is said to appear under microscopic examination to resemble a silica-filled foam, (6).

(2) The author has made extensive observations on hyalite opal from Mexico which displays play-of-color when sliced thin and observed by transmitted light and minimal play-of-color by ordinary observation. Evidence is seen that gas (presumably carbon dioxide) was being evolved in and around the gel mass up to the time the gel set. The gas was trapped at varying stages of its movement in and around the gelling mass.

This is incompatible with the sedimentation hypothesis. It supports the proposition that the lattice structure was established *after* the mass gelled.

(3) The sedimentation hypothesis appears to be untenable for other reasons. A steady state process is invoked requiring more than a million years of balance between several critical factors. One of these is the sedimentation of only one size of silica particles—in the case of precious opal—particles of other sizes being rejected and not accumulating to disrupt the process further. Alternating repeatedly with this process is one in which many sizes and shapes of particle sediment together, also forming a close-packed mass, (2) the septa between looking much like the septa between the gas cells in a foam.

(4) Natural products related to gelled silica and to progressively silicified silica gel have been reported, (7). These include silica gel and opalites or opaloids. The degree of silicification of the gel is indicated by the varying ability of the product to take up water and the initial and final properties such as density and refractive index.

(5) An as yet secret process exists by which an artificial opal is made (8) resembling natural precious opal in many properties including play-of-color and a lattice structure similar to natural opal (5). The process is obviously achieved in a "short" time, contra-indicating the involvement of any process analogous to the sedimentation hypothesis. A "short" process is not necessarily incompatible with the operation of the foam hypothesis.

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Environmental Geology of Vigo, Clay, and Sullivan Counties, Indiana

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Purpose and Scope

The purpose of this report is to make available to regional, county, and city planning groups a study upon which they may base future decisions for land uses in their respective jurisdictions. This study will also aid those local, state and federal agencies which need recent data on various geologic aspects of the counties for ground water studies, landfill site proposals and flood insurance.

The scope of the report is limited to the geologic aspects of the region and does not attempt to assess the biological factors of the environment. The main sources of information for this report came from Montgomery *et al.* (3), Kelly *et al.* (2), Jones *et al.* (1), and from water well records on file at the Water Resources Division of the Indiana Department of Natural Resources.

Setting and Geology

Vigo, Clay, and Sullivan counties are located in southwestern Indiana along its border with Illinois. The topography is one of rolling hills and numerous river valleys. Most of the streams are small to medium sized and are part of the Wabash River drainage basin.

The Wabash River flows through two of the counties (Vigo and Sullivan) and, for a portion of its length, is the Indiana-Illinois boundary. The bottom lands and upland terraces along the Wabash are extensive and the flood cycles of the river have greatly influenced the landscape along its course. Generally the river valley is wide with gently sloping banks. In some areas the valley becomes narrow. These are the areas where the river has cut into the bedrock.

The whole area has been glaciated. The glacial cover consists mainly of Illionian age till with a small area in northwestern Vigo County covered with Wisconsin age ground and end morainal materials (5). The bedrock is Pennsylvanian shales, sandstones, limestones and some extensive coal seams. The dips are generally shallow and trend to the west or southwest (4). The area is along the eastern edge of the Illinois Basin and open pit coal mining is an important industry. In the past there were a number of underground coal mines but these have been abandoned.

The present drainage appears to roughly follow the pre-glacial drainage patterns. (Fig. 1) illustrates the bedrock topography. The Wabash River follows the bedrock valley of the pre-glacial river. Also, the general course of the Eel River in southern Clay County parallels a bedrock valley which drained to the southeast. The present Eel River travels southwest, also, to flow into the White River in Green County south of Clay County. Busseron Creek southeast of

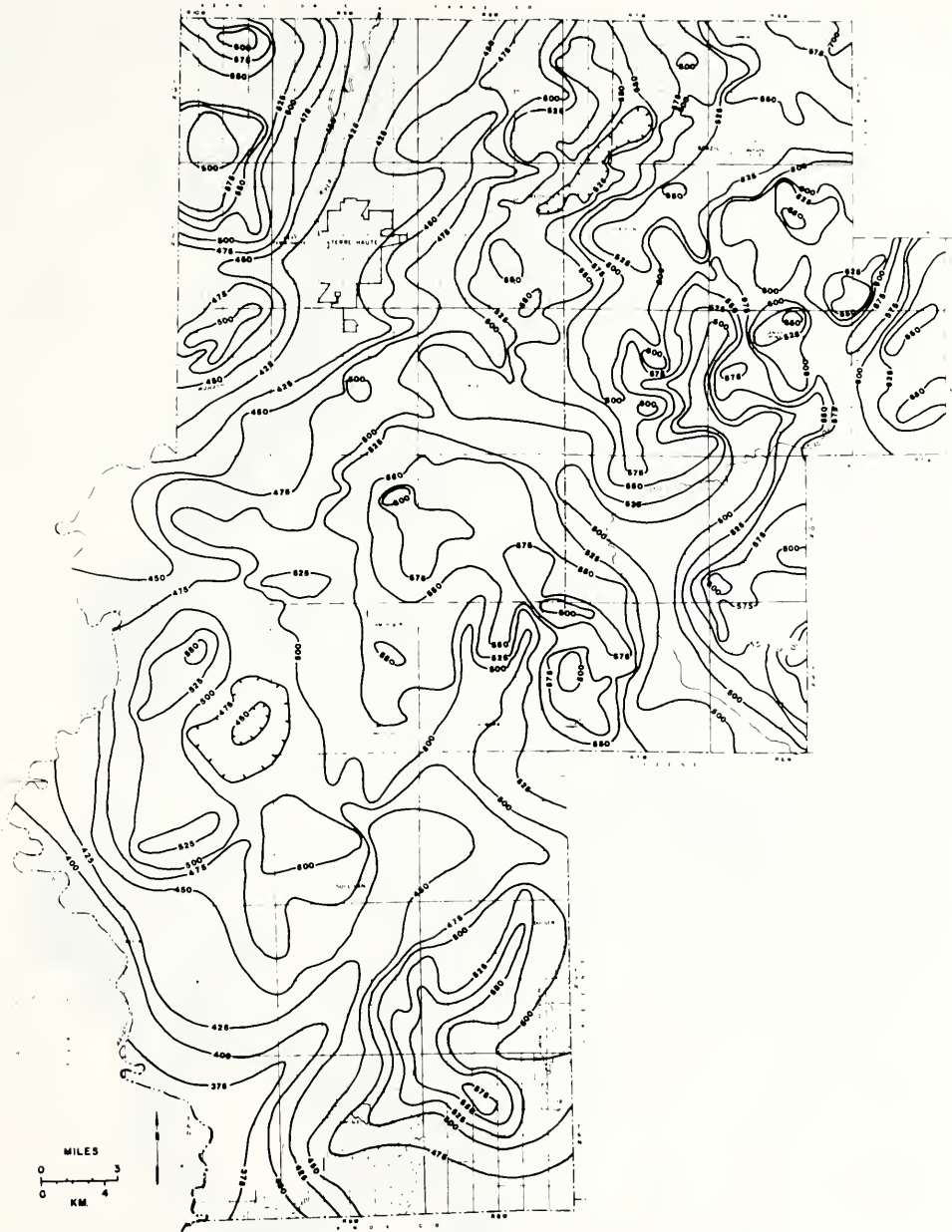


FIGURE 1. *Bedrock surface in Vigo, Clay and Sullivan Counties. The elevations are in feet above sea level.*

Sullivan follows a third bedrock valley to flow into the Wabash near the southwest corner of the map.

(Fig. 2) illustrates the drift thickness in the region. There are extensive areas which are covered with less than thirty feet of unconsolidated material over the bedrock. Site surveys and test drilling would be advised for these areas before developments requiring extensive excavating are started.

Water wells in these areas are more likely to be in the bedrock rather than the sand and gravel layers of the glacial till. The one exception to this will be those wells on the flood plain of the Wabash River. The most productive wells are located in the sand and gravel seams rather than the bedrock. None of the



FIGURE 2. *Thickness, in feet, of glacial drift covering Vigo, Clay and Sullivan Counties.*

wells in the area are large water producers. The flows range from less than two gallons per minute to as much as fifteen gallons per minute for a four inch diameter (or equivalent) well. A flow rate of less than ten gallons per minute to as much as fifteen gallons per minute for a four inch diameter (or equivalent) well seems to be typical of the area.

There are some dry wells scattered through the area with no apparent pattern. It is recommended that test wells be drilled before buildings are erected. It is a possibility that there may be no obtainable ground water at a given site. Industrial sites should be developed in those areas which have thick deposits of glacial cover. The reasons are that bedrock will present no problems in excavating for the building footings and the increased likelihood that sand and

gravel seams will be encountered when drilling water wells. An alternate solution for industrial water supply is to use the surface water from the Wabash River.



FIGURE 3. Flood-prone areas, shown in black, of Vigo, Clay and Sullivan Counties.

(Fig. 3) outlines the flood-prone areas of the region. It should be noted that industrial plants can be located, in some areas, quite close to the Wabash River with little danger of being flooded. Therefore it is possible for river water to be piped to those plants rather than having to depend upon ground water for their operations. The flood-prone areas were mapped by using the soil surveys. Some soils develop only on flood plains and so are excellent indicators for the extent of probable flooding of a river. The soils used to outline the flood-prone areas are, Armiesburg, Atkins, Cuba, Eel, Genesee, Holly, Petrolia, Ross, Sharkey, Shoals, Sloan, Stendal, Wakeland, Waverly, Wilbur, and Zipp.



FIGURE 4. *Elevation, in feet above sea level, of the ground water pressure surface in Vigo, Clay and Sullivan Counties.*

(Fig. 4) shows the ground water pressure surface elevation above sealevel in the area. The ground water flow generally follows the bedrock valley system developed by the pre-glacial drainage. In most wells, the water table is in a range of fifteen feet to over eighty feet below the ground surface. There are some dry holes in the region. However, it is likely that the water table has been penetrated by these wells but the flow rate into the well is so low that they were classified as dry by the driller on his log.

The ground water flow is toward the three main rivers of the area—Wabash River, Eel River and Busseron Creek. The only exception to this is an area immediately south of Brazil. There are strip mines in this area and it appears that

the pressure surface has been lowered by mine dewatering operations. There are numerous strip mines in the region but the pressure surface only reflects this one artificial lowering. It appears that the coal mining in the area has had little effect on the water table in the three counties. This does not mean that some wells close to the excavated areas might not be affected. Only, on a larger county-wide scale, there is little evidence of permanent changes in the pressure surface due to mining activities.



FIGURE 5. *Suitability of soils in Vigo, Clay and Sullivan Counties for septic tank filter fields. The white areas have acceptable soils and the black areas have unacceptable soils.*

(Fig. 5) shows areas which would be suitable for large subdivisions that use septic systems as the means of waste treatment. There are many sites which would be acceptable and also many others which are not acceptable. This map was based upon the soil types found in the region. Acceptable soils are those

which have fair to good drainage (but not too rapid) and are thick enough so there is no danger of contaminated water reaching the bedrock to mix with the ground water. This map should be used to judge whether or not large subdivisions might better have a central waste treatment plant rather than depending upon septic systems. It is not meant to judge the adequacy of an individual septic system. Septic systems in high-clay soils can work if the finger system is long enough. But lots in many subdivisions are one-half acre or less and this may not be adequate where fifty or more families have homes on less than forty acres of land.

The following soil types are acceptable for septic systems in high density population areas: Alford <12% slopes, Ayrshire, Camden, Crane, Elston, Fincastle, Fox, Iona, Iva, Markland <12% slopes, Muren, Parke <12% slopes, Princeton <12% slopes, Proctor, Reesville, Russell <12% slopes, Tippecanoe, Warsaw, Whitaker, and Xenia.

The following soil types are judged to be inadequate for the proper functioning of septic systems in subdivisions. Excessively fast drainage is encountered in Ade and Bloomfield soils. Very poor drainage is found in Ava, Bartle, Carlisle, Cincinnati, Cory, Henshaw, Kings, Lyles, McGary, Millsdale, Patton, Ragsdale, Rensselaer, Vigo, Vincennes, Washtenaw, and Westland soils. Thin soils with bedrock close to the surface are Randolph and Shadeland. Soils developed on steep slopes are Alford >12% slopes, Gibson >12% slopes, Hennepin, Hickory, Markland >12% slopes, Negley, Parke, >12% slopes, Princeton >12% slopes, Rodman, and Russell >12% slopes. Soils which develop on flood plains should be avoided since they are subjected to periodic flooding. These soils have already been listed in the discussion of (Fig. 3) flood-prone areas.

(Fig. 6) illustrates areas which are suitable for sanitary landfills. For a landfill site, a number of stringent criteria must be met. The foremost is that there should be no likelihood that any leachate will leak from the landfill to contaminate surface waters or any useable supply of ground water. Such a condition of total confinement of leachate can only be achieved in a large body of clay or silt. The potential site must also not be in a flood plain with any surface water running through it. Lastly, the site should be reasonably close to the cities which will use the site. Long distance transport of waste becomes too expensive for municipalities.

The sites in (Fig. 6) are divided into two categories. The best sites contain fifty feet of clay measured from the surface downward. The acceptable sites contains thirty feet of clay measured from the surface downward. It is assumed that the trench method of landfill will be used. The trenches should be no deeper than twenty feet which will provide an unbroken bottom seal of at least ten feet to contain any leachate.

These are recommended localities based upon the clay content of the area. It will be necessary to do a site study at a particular location before a final decision is made to use the area for a sanitary landfill.

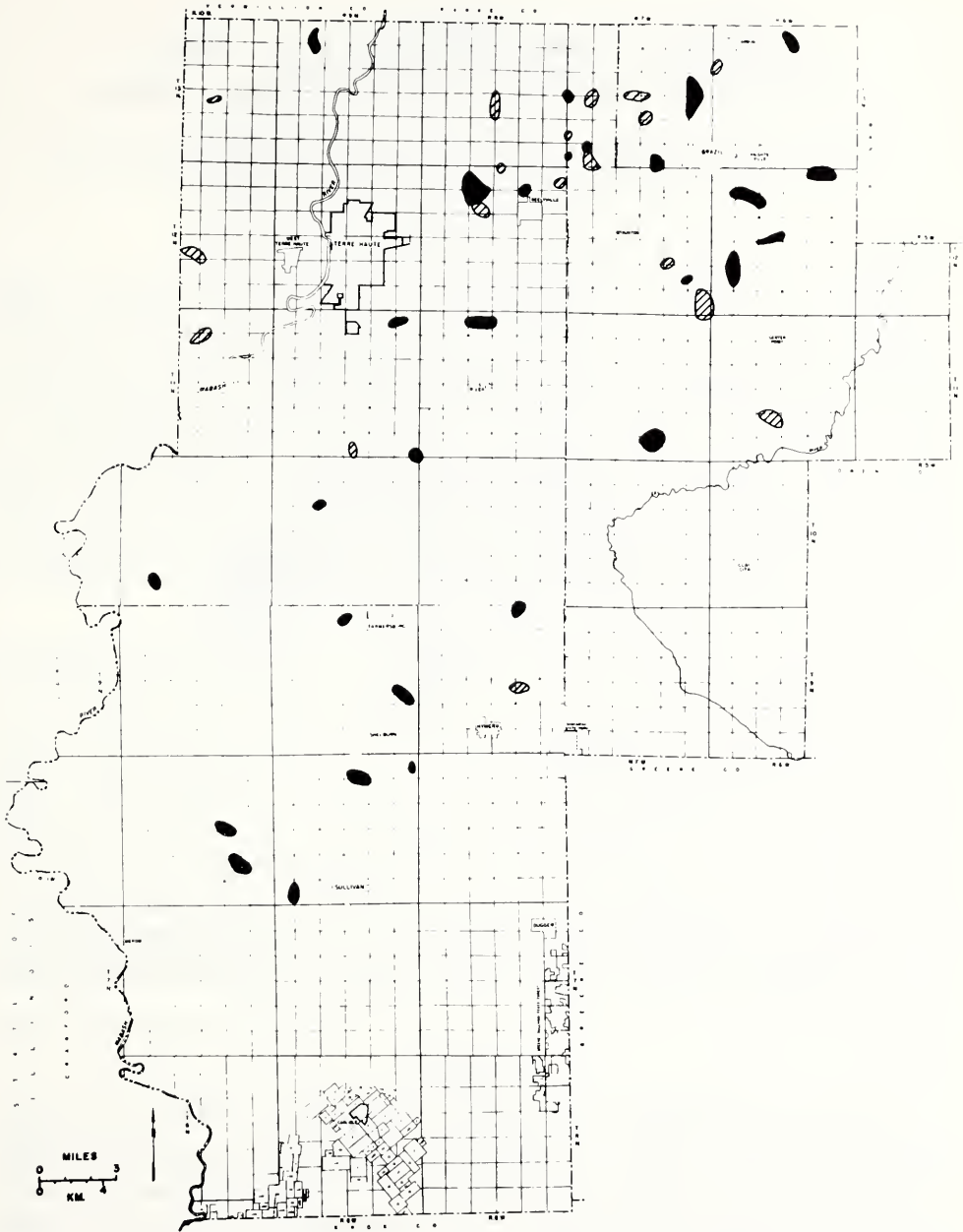


FIGURE 6. Areas in Vigo, Clay and Sullivan Counties which are potential sites for sanitary landfills. The black areas contain thirty feet of clay and the stripped areas contains fifty feet of clay.

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An Example of Primary Sedimentological Control of Sulfur Distribution in Coal V in Sullivan County, Indiana

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Introduction

Since the advent of serious ecological concern over emission of sulfur oxides from combustion of coal, interest in sulfur distribution in coals has been growing. Pennsylvanian coals of the Western Interior Basin of Iowa, Missouri, Kansas, and Oklahoma are especially high in sulfur because of the dominance of marine conditions as evidenced by extensive limestone development. In contrast, the Appalachian Basin coals near source areas of Pennsylvanian sediments are low in sulfur. the sulfur content of the Illinois Basin coals on the average is intermediate to these extremes, but wide variations in sulfur content exist between seams and locally within seams depending upon conditions of deposition of the sediments.

A common Midwestern roof condition is that of a black shale and limestone marine association. The marine environment is responsible for a higher concentration of sulfate ions. The most important aspect to our discussion of the black shale environment is its reducing conditions which resulted in a concentration of sulfides. Zangerl and Richardson (1963) extensively studied the character and variability of some of the black shales in Indiana. This depositional situation has generated only high sulfur coals ($>3\%$ sulfur). Indiana coals IIIa (Colchester), IVa (Houchin Creek-Summum No. 4), V (Springfield), Va (Briar Hill), and many other seams in the Upper and lower Pennsylvanian commonly have the marine limestone and black shale roof.

Methods

The present investigation near Sullivan, Indiana (Fig. 1) is concerned with a clastic wedge (Fig. 2) from a "prograding" fluvial-deltaic crevasse splay deposit that has prevented sulfide contamination of Coal V from marine sulfate solutions associated with deposition of a "transgressing" unnamed black shale and the Alum Creek limestone (Fig. 3). The coal immediately surrounding the deposit averages 3.5-4.5% sulfur whereas the thick clastic wedge has maintained values of 0.5 to 1.0% sulfur in the underlying coal. This coal deposit contains some of the highest quality coal in the Midwest with respect to sulfur content, ash content, and coal thickness (USBM, 1927). Specific coal analyses with good map control are not available at this time, unfortunately. Most of the drill data was in the form of coal drilling logs made by one driller over about a 40-year period. Electric logs are rather limited for the study area and some begin deeper than the intervals under consideration. All log information was digitized for machine processing. CALCOMP plotted output was manually contoured for economy.

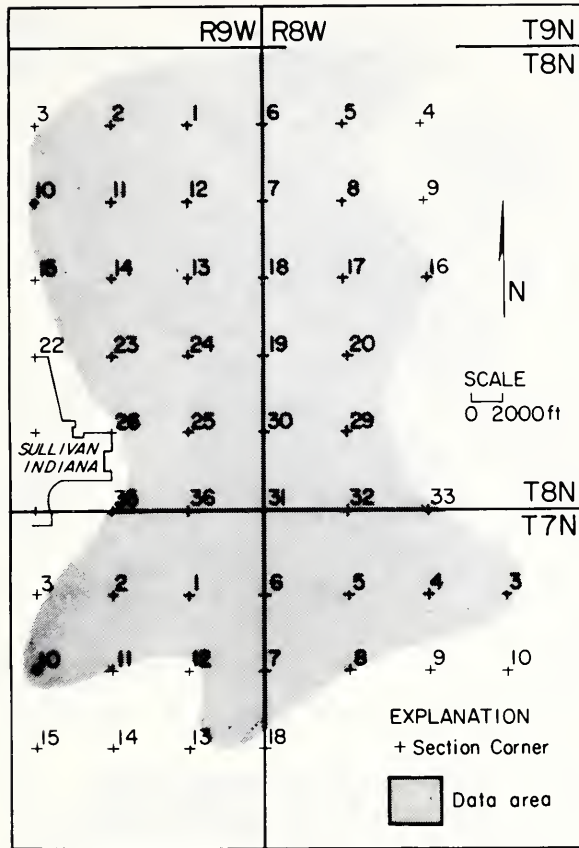


FIGURE 1. Study Area Base Map

Low Sulfur Association

Coal V is overlain at the extremities of the deposit by black shale and limestone. There is a clastic (sandstone and shale) wedge overlying Coal V in the center of the area. The wedge is asymmetric with respect to a coal absent channel area. The Alum Creek limestone (Fig. 3) and black shale rise up over the wedge, thin, and then pinch out as the clastic wedge (Fig. 2) thickens toward the channel. The distribution of the overlying Coal Va (Briar Hill?) has also been affected by the wedge. Indiana Coal VI and the underlying limestone (Providence-Brereton) and black shale (Anna) were not affected greatly by the presence of the clastic wedge. This would imply that the wedge had subsided to form a new "base level" by the time that the Anna shale was deposited.

Most shale splits in the coal have limited distribution and are associated with a coal absent sandstone channel area. Because the splits are within the coal, it is thought that the channel existed concurrently with coal deposition.

In many areas there are two horizons of the Alum Cave with a light colored claystone or laminated shale between. Usually there is an unnamed black shale underneath the limestone. Frequently the limestone, black shale, and light colored shale are intercalated so that various vertical sequences of the beds exist. It appears that either there may have been some reef-like thickening of the limestone on the edge of the clastic wedge or the limestone was thickened by dilution with argillaceous clastics. It is evident that the limestone distribution was controlled by the clastic wedge.

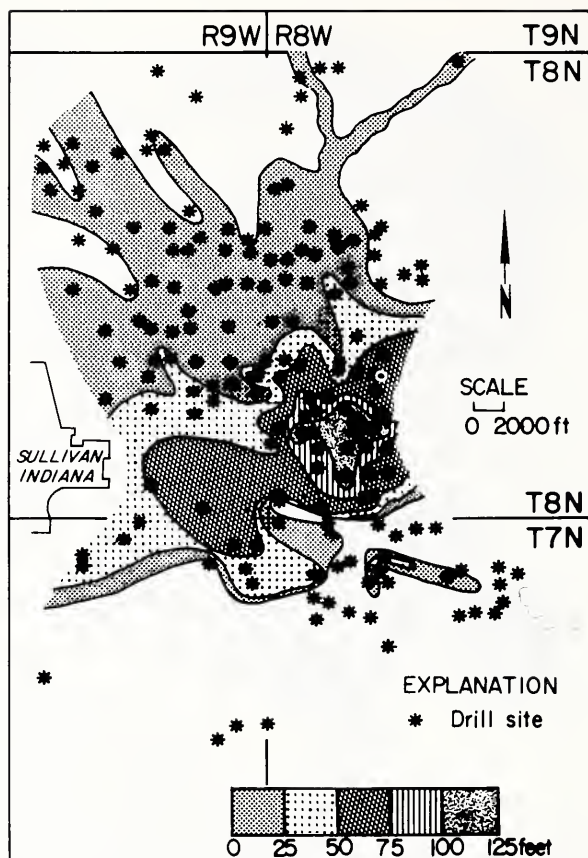


FIGURE 2. *Thickness Map of Clastic Wedge over Coal V*

(Fig. 2) shows the distribution and thickness of light colored clastics between Coal V and either the Alum Cave limestone, associated black shale, Coal Va, or Anna Shale, whichever was first encountered above. Other sand units exist overlying the Alum Cave and Coal Va. In some cases it is not possible to absolutely distinguish these units from the clastic wedge. However, the clastic wedge continued to be deposited subsequent to cessation of Coal V accumulation. It is possible to consider the clastic deposition as a semi-static "prograding" deltaic finger. On a regional scale this crevasse splay deposit is a very localized radial form. However, the contour lines indicate that the full extent of the wedge has not been mapped because of limited data. In other known areas each splay deposit is only a part of a much larger fluvial-deltaic system. Overall the stratigraphy is similar to the model described by Gluskoter and Hopkins (1970).

Another aspect of the clastic wedge noted on drillers' logs, which is not detected on available electric logs, is the presence of a thin "rash" or black shale between the coal and the light colored clastic wedge. Apparently this black shale originated in quite a different environment than the black shale below the Alum Cave limestone. Donald Eggert of the Indiana Geological Survey (oral communication, 1977) has located what may be a similar "rash" condition over a relatively low-sulfur coal at the Park Coal Company surface mine in Pike County, Indiana. There terrestrial plants and vertical tree trunks are associated with the rash. Also, there is a split in the coal, inferred to be a levee overbank

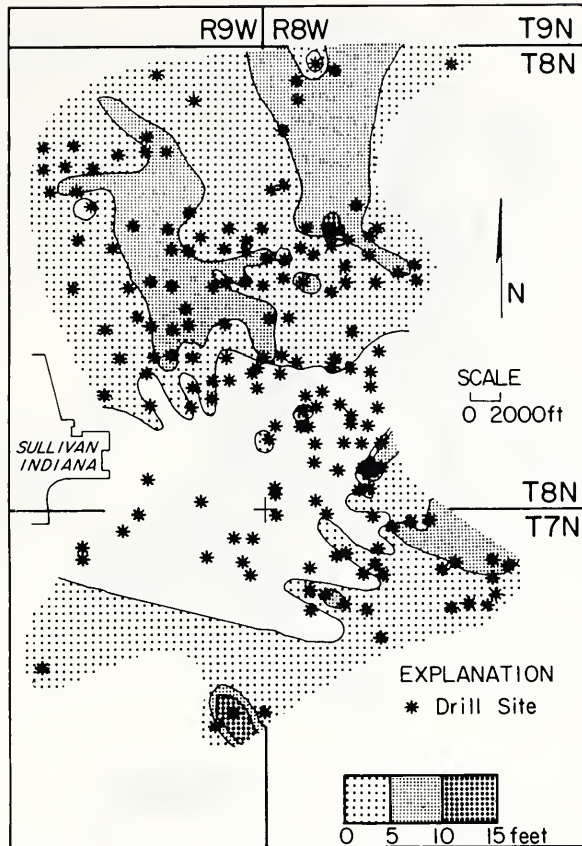


FIGURE 3. *Thickness Map of Lower Alum Cave Limestone*

deposit, which widens to 25 feet on the high wall face. No such overlying “rash” occurrences have been noted in other Illinois Basin studies.

Facies Analysis

A digital facies analysis was made of drillers’ and electric logs for the interval between coals V and VI. On an isopach map separation between these two coals varied from 31 feet to 137 feet. Maximum separation coincides with the area of maximum clastic wedge thickness. Contoured percent sand in the V to VI interval (not illustrated) suggests a dendritic pattern with sand occurring in channels and subsiding pockets and low shaly areas occurring between the sandy areas as interdistributary bays. Additional facies analysis was done at other intervals and horizons.

Coal Thickness

The area of maximum Coal V thickness (Fig. 4) corresponds with the area of maximum clastic wedge deposition. In some localities the coal gradually thins toward the channel area but in others the coal first thickens in a ridge form near the channel then abruptly thins. This is probably related to levee patterns of the channel complex. On cross sections there are several instances where Coal V thins over thick sediment intervals between coals V and IVa. The area of maximum Coal V thickness also corresponds with an elevation low (syncline) relative to the surrounding coal elevation surface.

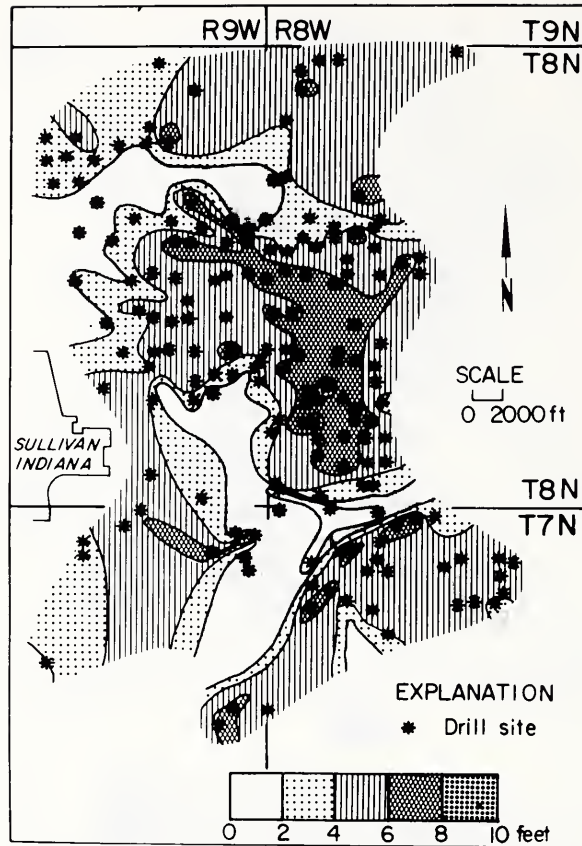


FIGURE 4. Thickness Map of Indiana Coal V

Coal thickness maps were also made for overlying coals VI and VII. In both cases clastic wedge and V thickness maps indicate little influence upon succeeding coal thickness. This would imply loading subsidence of Coal V and the clastic wedge before Anna Shale deposition. Coal VI thicknesses are erratic in contrast to the rather broad contour spacing for Coal VII thickness.

Conclusions

The low-sulfur coal occurrence in the Indiana coal V seam near Sullivan, Indiana originated by virtue of a synchronous and subsequent accumulation of fluvial-deltaic crevasse splay clastics within and over the coal bed. The clastic blanket isolated the coal locality from marine sulfate contamination. The clastic wedge had a notable effect on the separation between coal V and the overlying coals, but had little effect on the thickness of those coals. The overall depositional pattern is similar to some of those found in Illinois with the exception of noting a black shale "rash" associated with part of the low sulfur coal area. Further studies of roof characteristics of Indiana coal seams probably will define additional low-sulfur coal resources.

Acknowledgements

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Abatement of Combined Sewer Overflow and Urban Runoff Pollution in Indianapolis, Indiana Using Deep Tunnel Conveyance and Storage

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Statement of the Problem

Most large metropolitan areas in the United States are served, at least in part, by combined sewer systems. Such a system combines both the storm water from urban areas during rainfall events with the domestic, industrial, and commercial sanitary waste water.

At normal and low flow periods combined sewers route all the waste water to the sewage treatment plant where it is treated and subsequently returned to surface streams. During rainfall events of high intensity, the sewer system's capacity to transport the combined storm and sanitary wastes is commonly exceeded. At these times the excess, untreated waste water is released into rivers and ditches from overflow structures of the combined sewers. This is judged to be one of the most serious water pollution problems in the area.

At the present time a major underground construction project is underway in the Chicago area to collect, transport and store the combined-sewer overflow for various parts of the city (2). An extensive appraisal of a similar system for Milwaukee is also currently under investigation (5). For Indianapolis the problem is less-well publicized because of the smaller extent of the metropolitan area, but because of similarities in topography and geology, similar schemes for correcting the problem apply. This paper is a preliminary analysis of the problem to underscore the impending need for a solution.

In Indianapolis, Indiana 140 combined-sewer overflow structures are present. It is estimated that 45% of the waterway pollution originates from combined sewer overflows (2). Four Indianapolis waterways receive combined sewer discharges: West Fork White River, Fall Creek, Pogue's Run, and Pleasant Run.

Curative solutions for the abatement of combined sewer overflows normally require substantial physical facilities and large capital expenditures. The Department of Metropolitan Development in Indianapolis (1) studied three curative solutions:

1. Complete separation of storm and sanitary sewer systems.
2. Treatment at, or near, individual points of overflow.
3. Collection of combined sewer overflow followed by treatment at one site.

The plan proposed in this paper for the elimination of combined sewer overflows is of the "treatment at one site" variety. Vertical shafts, as depicted in (Fig. 1), located near the overflow points would be used to collect the waste water and drop it into tunnels which would convey the captured water to a subsurface storage reservoir. From this location, the polluted water would be

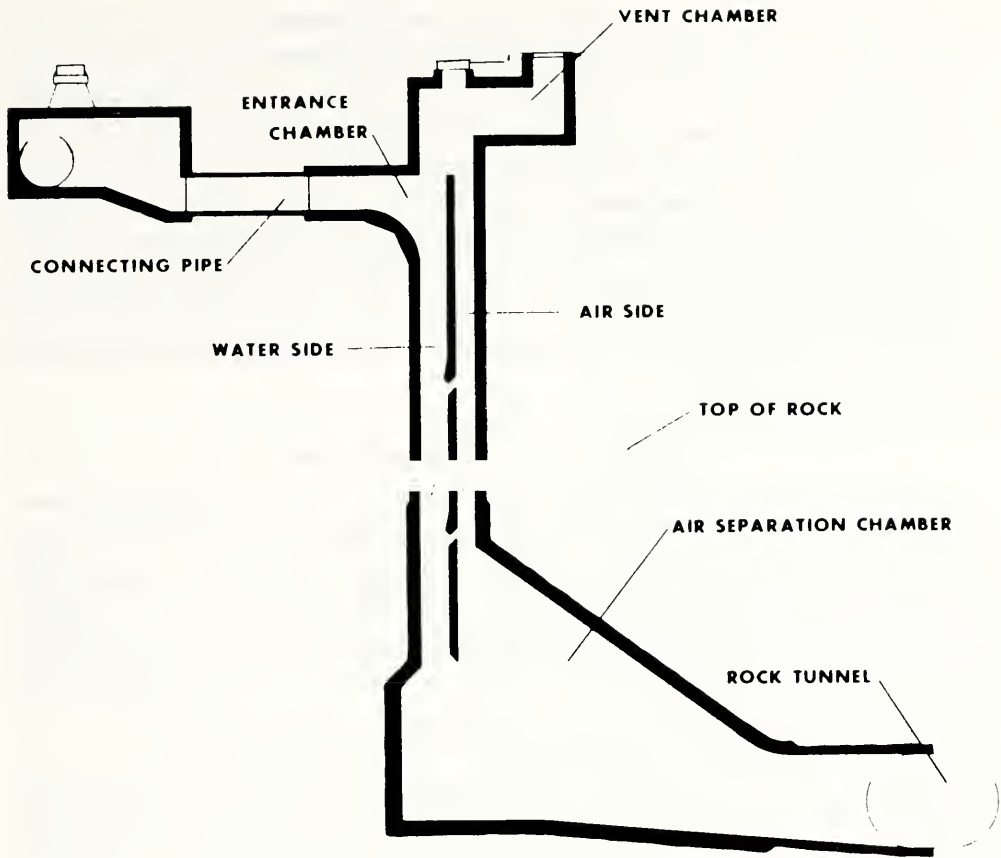


FIGURE 1. Cross-section of a typical dropshaft structure.

pumped during dry periods to the surface sewage treatment plant for proper treatment prior to entering the waterways. This would also provide a constant flow of waste water through the treatment facility which would optimize efficiency.

Geology and Hydrology of the Study Area

Because the proposed structures for collection, conveyance, and storage of the combined sewer overflows are all underground, a detailed knowledge of the soil, bedrock and ground water conditions for the area is necessary to insure success of this plan.

The surficial geology of Marion County is primarily a consequence of the several continental glaciers that covered the county during the Pleistocene epoch. These deposits consist primarily of till plus glacial outwash, the latter associated with the major streams in the county. The thickness of the glacial drift in the study area averages about 100 feet.

The bedrock formations lying immediately below the glacial drift in Marion County consist of a series of limestones and dolomites of Silurian and Devonian age in the central and eastern parts of the county, and shales and sandstones of Devonian and Mississippian age in the western part. These rocks dip gently to the southwest at a rate of 20 to 30 feet per mile with no major faults or deformations present.

In conveying and storing wastewater in underground structures, great care must be taken not to pollute the ground water. The potential for ground water contamination in the glacial till is much less than in the extensive sand and gravel deposits (outwash material) adjacent to White River and Fall Creek. The uppermost limestone and dolomite units are quite permeable within the first 100 feet of the bedrock surface, and thereby are susceptible to pollution. This high permeability is due, most probably, to solution channels which formed when the rock was exposed during pre-glacial times. Permeability of these limestone and dolomite units is greatly reduced in the western and southern parts of the county where they are overlain by the younger shales of Devonian and Mississippian age.

Hydrology of the Proposed Plan

An accurate knowledge of the quantities and rates of combined sewer overflow is vitally important to the development of this underground conveyance and storage plan. The storage capacity of the underground reservoir was designed for a year of high rainfall activity. In 1957 the Indianapolis area received approximately 55 inches of precipitation compared to the normal annual amount of 39 inches. In this study it was assumed that a day having greater than 1/4 inch of rainfall would produce overflows from the combined sewers. Fifty-nine days of greater than 1/4 inch of rainfall occurred in 1957. On days of greater than 1/4 inch rainfall, it was assumed that 50% of the rainfall for the area of combined sewers in Indianapolis would infiltrate into the ground or run off into streams, 25% would travel through the combined sewers to the sewage treatment plant, and 25% would overflow from the combined sewers into surface waterways. It is this last 25% of the flow that would be captured and stored by the proposed underground system.

(Fig. 2) shows the storage requirements of the underground reservoir in 1000's of acre-feet as related to different pumping rates to the treatment plant throughout the 1957 design year. As seen from the figure, higher pumping rates result in lower storage volume requirements. For a pumping rate of 125 cubic feet per second (cfs), a storage volume of 2250 acre-feet would be needed. This pumping rate would empty the reservoir each year without permitting a carry

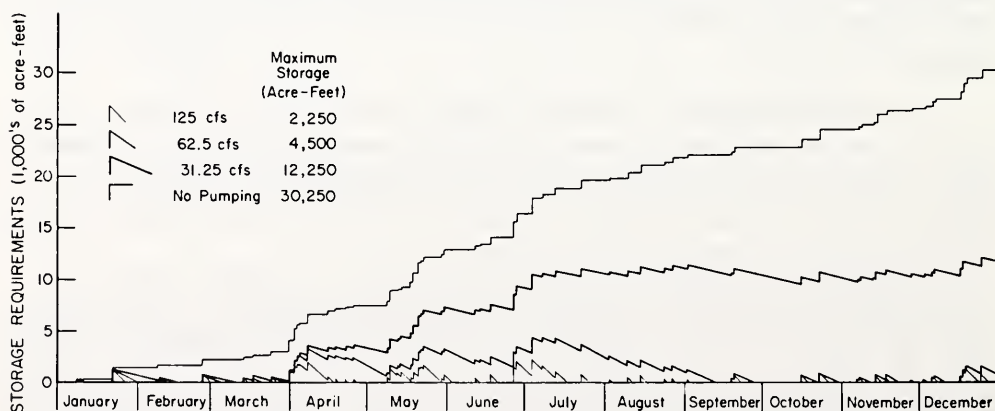


FIGURE 2. Storage requirements of combined sewer overflows for 1957 rainfall year considering various pumping rates.

over of one year's wastewater to the next. Such a carry over would reduce the reservoir's storage capacity for the following year's run off and hence be undesirable.

In order to calculate the tunnel distance, it was necessary to determine the surface area served by the combined sewers within the drainage basins for those streams in Indianapolis which experienced overflows. This information was used to determine the volume of wastewater that would be dropped to and conveyed through the tunnels beneath each stream. The size of the tunnels to convey these wastewater flows is related to the velocities that can be attained in the tunnel. In rocks similar to the carbonates beneath Indianapolis, Harza Engineering in a study for the Chicago area (3) estimated that for a 10 year return period storm, the safe maximum velocity in a moled, unlined tunnel would be 28 feet per second (fps).

The Indianapolis tunnel system was designed to accomodate the discharge rates of a 10 year return frequency storm with a 30 minute duration. Table I summarizes the maximum diameters of each waterway tunnel needed to provide for such rates of flow. The calculations used to estimate the volumes required for the storage reservoir and tunnel diameters, velocities, and slopes are developed by Worland (6).

TABLE I *Diameters of moled, unlined tunnels beneath waterways.*

Waterway	Diameter (ft)
White River	19.0
Pleasant Run	17.1
Fall Creek	14.3
Pogue's Run	9.2

Engineering Geology and Construction

Grouting the surrounding rock and lining of the tunnels are methods used to reduce seepage through the tunnel and reservoir walls. If such seepage is outward, it can pollute the aquifer, and if inward, the effect would be a loss of ground water and an increased volume of water for treatment. Grouting consists of injecting a mixture of cement and water into the rock to plug water-transmitting passages. Grouting would be used as the major deterrent to seepage in the structures proposed in this paper. Observations of completed tunnels in similar rock beneath Chicago, Illinois indicate that grouting can reduce infiltration to about 0.05 million gallons per day per mile of tunnel (3).

Dropshaft construction in the glacial deposits would involve the use of either soldier pile and lagging, or steel sheet pile supports depending upon the depth and characteristics of the soil. Excavation through the bedrock would be performed by either drilling and blasting or raise borings. The raise boring technique involves the drilling of a 12 inch pilot hole into a prepared chamber at the base of the shaft and upward reaming of the hole, in one or more passes, until its full size is reached.

The 27 miles of tunnels proposed for the Indianapolis plan are to be excavated by tunnel boring machines (TBM) and left unlined with rock bolts

used for crown support. Drill and blast techniques would be used to excavate transition sections between different size tunnels and to excavate the initial reach of each tunnel and thereby provide an assembly chamber for the TBM.

For the mined storage reservoir (or chamber) it is anticipated that the room and pillar mining techniques would be employed, creating a comparatively close spaced, mined network of passageways as displayed in (Fig. 3). Room sizes would be 35 feet wide by 50 feet high with pillar sizes being 100 feet square. A grout curtain would be needed to encase the reservoir in order to prevent outward seepage and thereby provide aquifer protection.

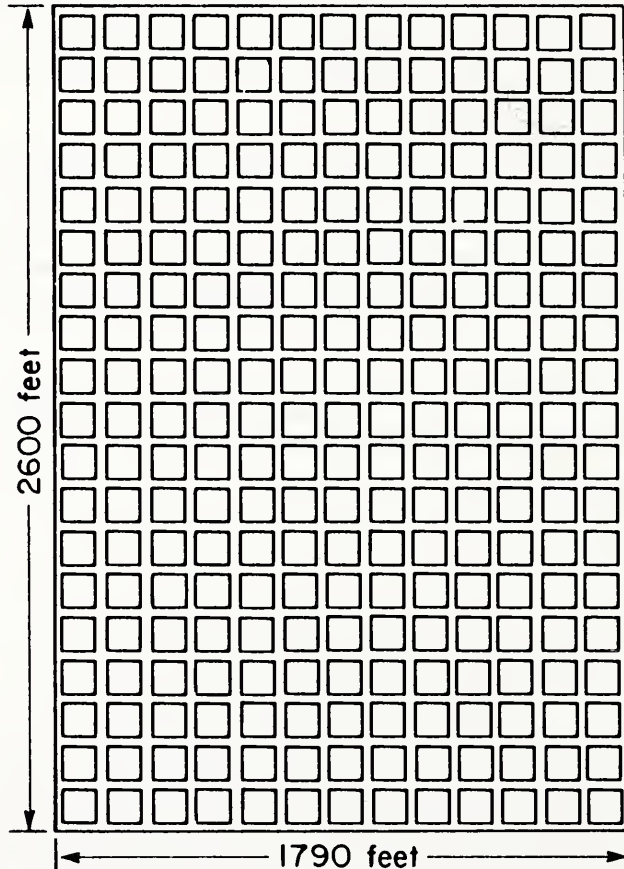


FIGURE 3. Areal view of 2,500 acre-foot storage reservoir with 100 foot square pillars.

The proposed storage chamber would require excavation involving conventional drill and blast techniques. The upper part of the chamber would be excavated as a large tunnel and the lower part, constructed by bench excavation which is essentially a quarrying operation. Smooth wall blasting methods would be required to minimize disturbance of the chamber arch and side walls. Rock reinforcement using rock bolts and shotcrete would be accomplished during excavation.

Overall Details of Project

Approximately 58 dropshafts located along White River, Fall Creek, Pogue's Run, and Pleasant Run would drop the overflows from combined sewers 250 feet vertically to conveyance tunnels located 75 to 150 feet into the

limestone and dolomite rocks of Silurian and Devonian age. These tunnels would transport the flow to a 2500 acre-foot mined out, room and pillar storage reservoir lying below a geologic section consisting of 100 feet of glacial drift, 75 to 100 feet of New Albany Shale, and 100 feet of limestone. The areal location of this reservoir would be immediately southwest of the junction of Meridian Street and I-465 on the south side of Indianapolis. From this reservoir the stored waste water would be pumped to the Southport sewage treatment plant for processing before it is released to surface waterways. (Fig. 4) is a cross-section of the main tunnel route beneath White River showing the tunnel orientations and storage reservoir location.

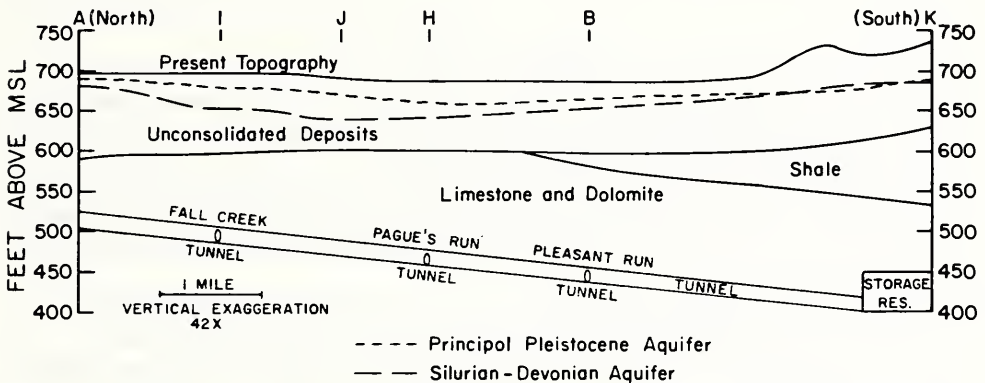


FIGURE 4. Cross-section of the tunnel and storage reservoir for area beneath White River.

The estimated total cost of such a project as shown in Table II is \$533,386,707 relative to 1979 figures.

TABLE II Estimated Total project cost (1979 dollars)

Tunnels	\$249,832,995
Storage reservoir*	104,901,325
Dropshafts	22,376,250
Pumps	8,840,000
Hardware	44,200,000
Subtotal	\$430,150,570
Subsurface exploration (10%)	43,015,057
Contingencies (8%)	34,412,046
Engineering, legal, administrative (6%)	25,809,034
Total Cost	\$533,386,707
Total minus cost of reservoir*	\$403,309,064

If it is economically feasible to develop the storage reservoir as an underground limestone mine as suggested by Hartke (Indiana Geological Survey, personal communication), then much of the expense of the storage reservoir would be removed from the project cost. This does seem possible as French and Carr (4) suggested some years ago that underground limestone mines in Marion County should become economical as crushed stone becomes more scarce in the area. The total cost for the project would then approach \$403 million which is economically competitive with the other methods of eliminating combined sewer overflows proposed for Indianapolis.

Conclusions

1. A major surface-water pollution problem exists in Indianapolis, Indiana because of overflow from combined sewers during periods of high rainfall intensity.
2. The problem could be solved by construction of an underground conveyance and storage system which would be located in bedrock to minimize ground water pollution.
3. Preliminary design calculations show the proposed system to be feasible and economically competitive with other methods for elimination of this pollution.

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Fracture Study of the Paleozoic Bedrock in East Central Indiana

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Introduction

In numerous regions, including East-Central Indiana, bedrock outcrops are restricted to isolated locations such as quarries, roadcuts, and streamcuts. Rock fracturing in such areas has often not been extensively studied and joint patterns are in many places unknown.

Understanding fractures and fracture patterns is useful for solving practical problems. The study of fractures is gaining significance with respect to investigating tectonic and non-tectonic phenomena, exploration possibilities, geologic history, and underground fluid flow. Such studies are proving to be valuable in the commercial development of groundwater, petroleum and mineral resources, and in land-use planning.

Throughout this paper, the terms "fracture" and "joint" will be used synonymously. All of the fractures considered in this investigation were vertical joints in horizontal bedrock unless otherwise noted.

General Geology

The study area is situated on the Cincinnati Arch, a positive feature trending north from Southwestern Ohio to the study region and then turning in a northwest direction across Indiana into Illinois (Fig. 1). The Cincinnati Arch has been eroded to expose the present-day outcrop pattern of Silurian and Ordovician bedrock with Devonian rocks dipping away on either flank. Bedrock exposure is usually masked by Pleistocene glacial drift. The Fortville Fault trends approximately N30E through the study area. It has been interpreted as a normal fault, downthrown to the southeast, with a vertical displacement of approximately 60 feet (8).

A feature interpreted as being a possible zone of structural inflection during the Ordovician (10), called the Hinge Line, can be traced through the southeastern part of the study area (Fig. 1). This line coincides with part of a larger structural alignment extending from the St. Lawrence River Valley along a branch of the Cincinnati Arch called the Findlay Arch in Ohio, to the New Madrid Fault Zone in Eastern Missouri. The Hinge Line may be a projection of the Grenville Front (11), which is the western boundary of the Precambrian Grenville Province exposed in Central Ontario.

Fracture measurements were taken at exposed bedrock locations in an eight county study area in East-Central Indiana. Orientation, persistence and intensity of samples of fractures were recorded at 11 quarries scattered throughout the area. The stratigraphy is summarized in Table I. Generally the

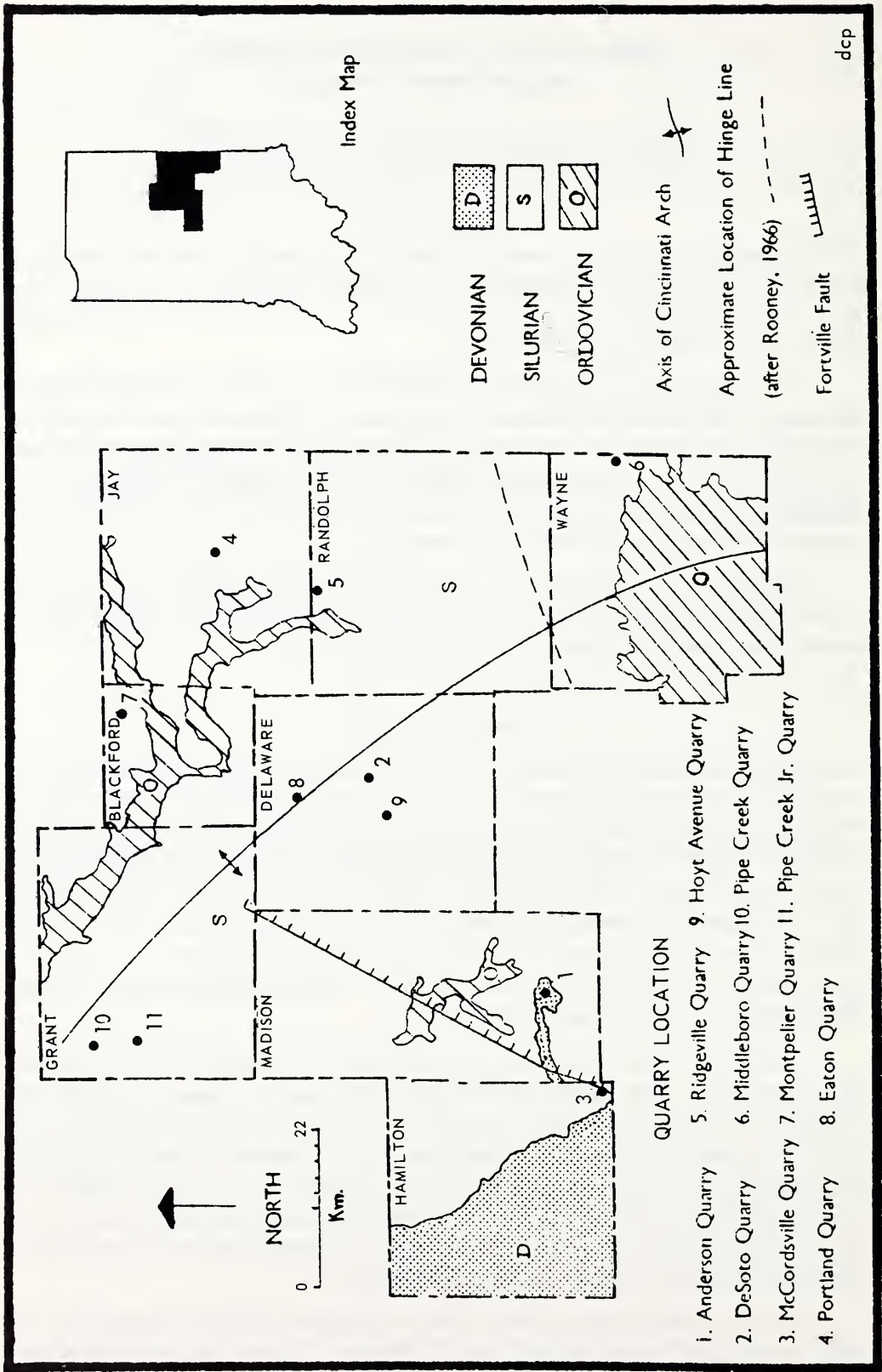


FIGURE 1. Map of study area showing bedrock geology and quarry locations. Three quarries in the Salamonie Fm. are found in the eastern three counties; four in the Louisville Fm. are in the center two counties; and three in the Wabash Fm. and one in the Jeffersonville Fm. are in the western three counties.

bedrock is dolomitic, formations are less than 100 feet thick, and bedding and parting are variable.

TABLE I *Stratigraphic Summary of Study Area*

FORMATION	AGE	ROCK TYPE	QUARRIES IN UNIT
Jeffersonville Formation	Dev.	Dolo.	1
Wabash Formation— Liston Creek Member	Sil.	Ls.	3, 12
Wabash Formation— Huntington Lithofacies	Sil.	Dolo.	11
Wabash Formation— Mississinewa Shale	Sil.	Sh.	
Louisville Formation	Sil.	Dolo.	2, 7, 8, 9
Waldron Shale	Sil.	Sh.	
Salamonie Formation	Sil.	Dolo.	4, 5, 6

The fractures present in the regional bedrock are not the nice clean fractures associated with tectonic activity. There are no conjugate sets or other evidences of shear. Fracture intensity varies considerably throughout individual quarries. The best fracture exposures are seen in quarries with several active faces available for study. Commonly the more pervasive fractures have undergone significant solution weathering. When the quarry is mined to these solution surfaces other fractures become less obvious. Distinguishing drill-hole blasting is not seen to confuse the pattern of observed fractures.

Method

In addition to recording strike and dip, a code was established for each fracture reading so that data could be sorted by the computer. The orientation of the quarry wall (or face) on which each fracture was observed was recorded as a code value 1 through 8 representing one of the eight 45° segments of the compass.

The vertical length, or persistence, of the fractures was taken into consideration according to the following classification:

1. Non-pervasive fractures: less than five feet in vertical length and usually confined to a single parting unit.
2. Semi-pervasive fractures: five to 15 feet in vertical length and often cut through several parting units.
3. Pervasive fractures: over 15 feet in vertical length and cutting through all observed beds and, in some cases, through different formations.

Spacing between fractures having similar orientation was designated as fracture intensity. Fractures were assigned an intensity code value of 1 through 7 if the spacing was less than 6 inches, 6 inches to 1 foot, 1 to 3 feet, 3 to 5 feet, 5 to 10 feet, 10 to 20 feet, or greater than 20 feet, respectively. A value of eight was assigned to those miscellaneous fractures that were not a part of any discernible set.

Fractures of varying persistence and intensity can be plotted separately and analyzed through the use of the computer program. Individual quarry faces or combinations of faces can also be plotted quickly in order to determine changes in fracture pattern observed throughout the quarry.

Statistical parameters may be used to decide whether a given fabric diagram is of random distribution, but the main feature which influences a structural geologist is the general appearance of a fabric diagram with respect to its overall symmetry and pattern. This investigation made use of Stauffer's (13) method for determining if samples are representative, but the determination of preferred orientation directions on the equal-area net plots were made visually.

Poles to planes are plotted on the lower hemisphere of an equal area net projection. A pole plots in a quadrant perpendicular to the strike in the direction opposite to dip. The poles of fractures measured for this investigation were plotted on a computer model of an equal-area net according to the Squared-Grid Method advocated by Stauffer (Fig. 2).

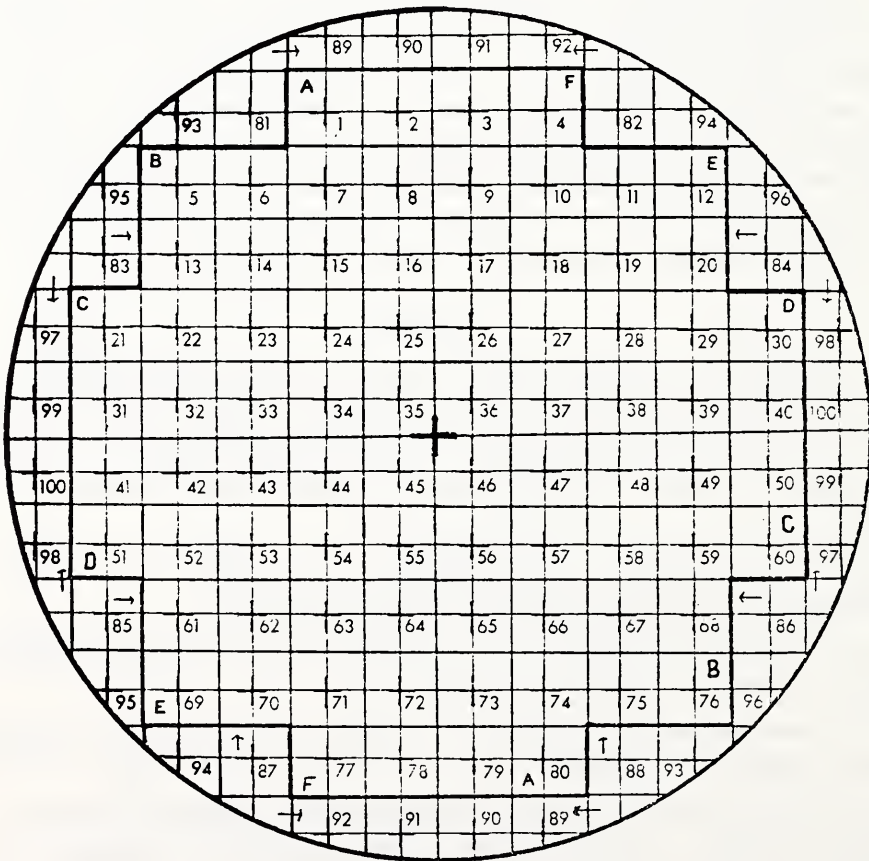


FIGURE 2. Double-grid system advocated by Stauffer (13) as utilized in a petrofabric data computer program developed by Samuelson (unpublished). The grid consists of one hundred 1% grid squares. A second offset grid is superimposed so the points can be counted twice for greater detail. The centers of one grid are the corners of the other grid. Cells on the periphery have counterparts on the opposite side of the circle, which are added together and count as single cells.

Results

(Fig. 3) is a display of the major pattern observed across the study region. The two maxima (N15W and N82E) are seen in all individual quarries. A minor set (N20E) is just barely evident. Three other minor sets which are to be discussed do not show on this summary plot because they are not seen in all quarries.

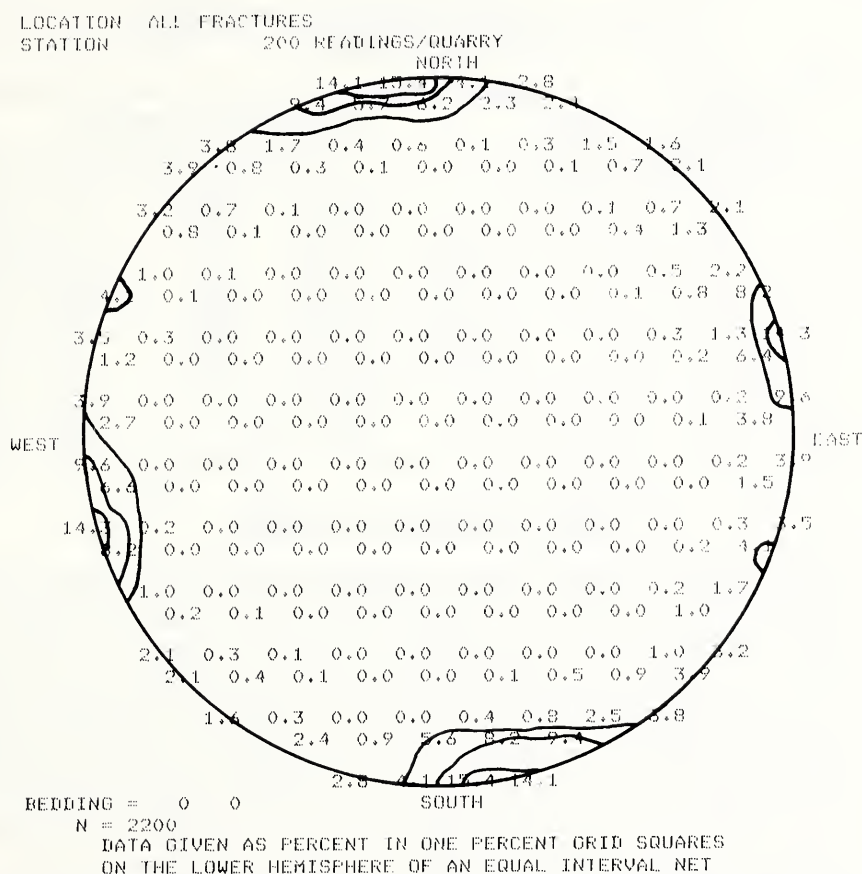


FIGURE 3. Combined plot of 200 readings per quarry taken from each of the eleven quarries; a total of 2200 readings. The two rows of data reflect the double grid count. All equal area nets are contoured at 4, 8, 12, 16, and greater than 20%.

Effect of Quarry Wall Orientation

The Irving Brothers Quarry, DeSoto, Indiana was examined initially and had the largest sample of observed fractures. Early in the study random samples of 75 fractures selected from the total were plotted and were shown to produce the same equal area net pattern provided the random sample data came from several quarry walls (6). Face orientation was a factor effecting observed fracture patterns in nine quarries. Each had individual faces that, when plotted separately, possessed variation in observed maxima compared to plots that took two or more faces of differing strikes into consideration. For this reason, an effort was made to examine at least two or more faces of differing strike at each quarry in order to prevent a particular fracture set from being overlooked. Regionally, the major two fracture directions did appear on faces of different orientation even though they had not appeared on all walls at each quarry.

(Table II). Apparently the inconsistency at each quarry is not necessarily due to parallelling a particular face because there were instances when perpendicular sets were not evident on the same wall. Their absence is probably due to local concentrations through the quarry. The minor sets listed in Table II were not seen at each quarry and therefore had less opportunity to be seen on faces of different orientation.

TABLE II *Observation on Different Quarry Walls
All Quarries Combined*

Wall	Number of Observations	Percent of Total	Observed Maxima Directions () = maxima less than 12%		
North	440	18	N14W	(N16E)	N80E
South	373	15	N16W		N85E
East	688	28	N15W		N78E
West	350	14	N14W	(N21E)	(N48E) N86E
NE	165	7	(N48W) N17W	(N20E)	(N77E)
SW	168	7	(N16W)	(N23E)	N81E
SE	105	4	N9W		N68E
NW	130	5	N8W	(N50E)	N87E

Effects of Fracture Persistence

When fracture persistence was considered at a particular quarry, all faces from which fracture readings were taken were included. Equal-area nets were generated for each of the three possible code values and compared to the total data plot for that quarry (6). The actual percentage of pervasive, semi-pervasive, and non-pervasive fractures varied considerably in individual quarries. At some sites a particular catagory was not observed. The maxima that were displayed by the various catagores varied considerably at individual quarries. Only plots of all fracture lengths observed in individual quarries were seen to be consistent with regional trends. However, when the three persistence catagories are summarized for the region the dominant two directions were seen in data for each type of persistence (Table III).

TABLE III *Variation in Maxima for Different Persistence Catagories
All Quarries Combined*

Persistence Catagory	Number of Readings	Percent of Total	Observed Maxima Directions () = maxima less than 12%		
All fractures	2419	100	N15W	(N20E)	N82E
Pervasive	264	11	N13W	(N15E)	N85E
Semi-pervasive	673	28	N15W		(N45E) N83E
Non-pervasive	1482	61	N17W		N83E

A major orthogonal fracture system had a set striking N82E and a set striking N5W. The angle between the sets of this system is 83°; the pervasive and semi-pervasive fractures displayed changes in abundance of the observed maxima. The regional trends may be seen in (Fig. 4). In the southwest part of the

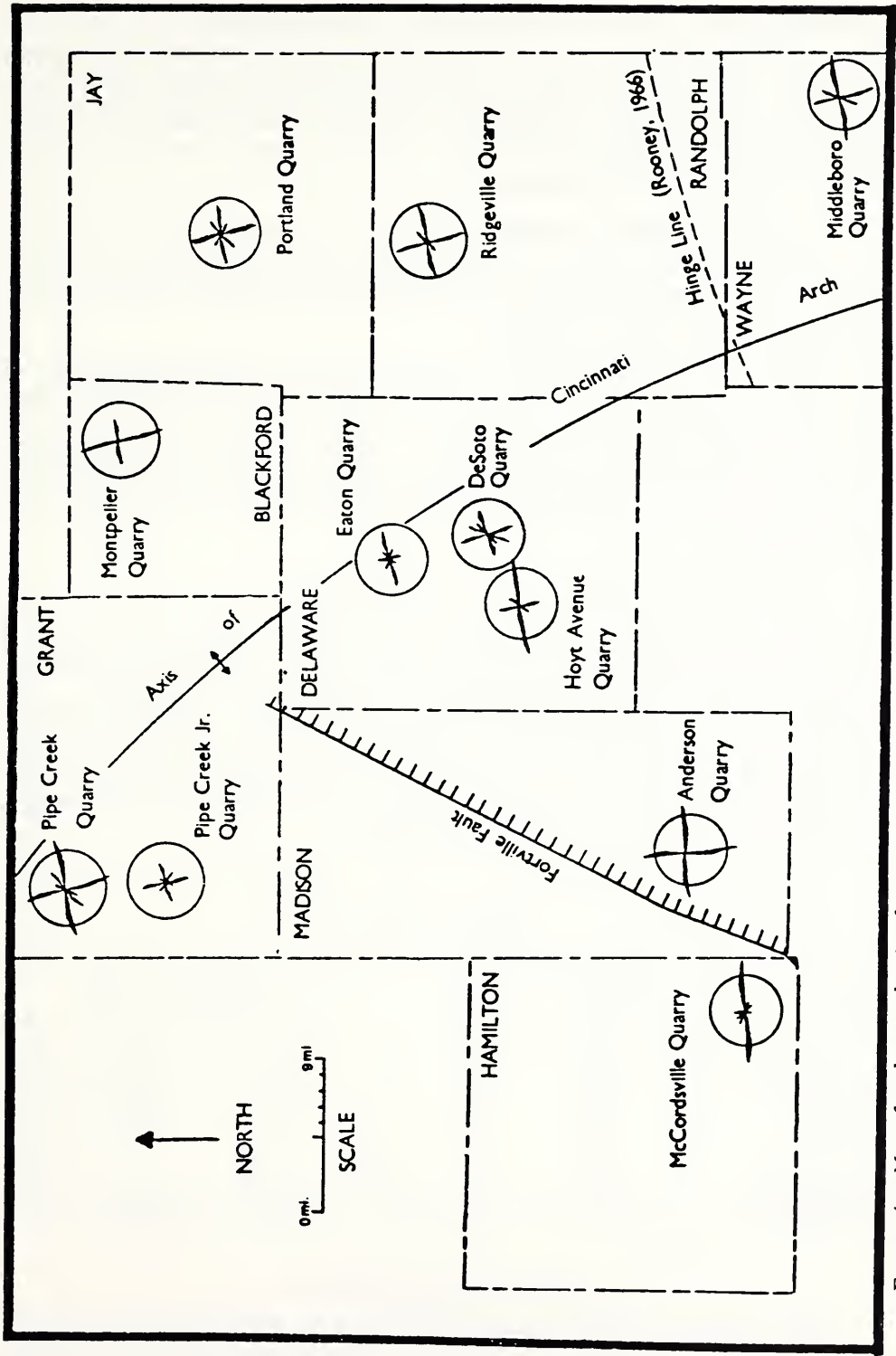


FIGURE 4. Map of study area showing fracture rosettes for all fractures. Circle represents 20% of the total sample for each quarry.

study area, the east-west set of the system is dominant except for the Anderson Quarry, Madison County where both fracture sets are approximately equal in abundance. It was also noted that no secondary sets were revealed at this quarry, regardless of the category of persistence considered. The stratigraphic position of the Anderson Quarry, which is the only quarry located in Devonian bedrock may be responsible for this deviation.

The quarries adjacent to and just east of the Cincinnati Arch axis show the two sets of the master system to be about equal. The exceptions are the Eaton quarry, where the east-west set is dominant and the Montpelier quarry where the north-south fracture set is dominant.

The pervasive fractures (11% of total observed) exhibit slightly different patterns (Fig. 5). The major north-south, east-west system still dominates with most of the secondary sets also being present. The Pipe Creek Jr. Quarry to the northwest is the exception because no pervasive fractures were observed there. This is the only quarry in which bedding dips are more than 5° (up to 30°) on the flank of a reef.

Effects of Fracture Intensity

The intensity (spacing) of fracturing in the Paleozoic rocks of East-Central Indiana varied little from quarry to quarry. There were no instances in which the complete range of fracture intensities were observed for a particular quarry. Most of the sets seen in each intensity category, however, possessed strikes that were identical, or nearly so, to those of the entire sample for that quarry (6). The summary of data for different intensity categories for the entire quarry sample shows consistency across the region (Table IV).

TABLE IV *Variation in Maxima for Different Intensity Categories
All Quarries Combined*

Intensity Category	Number of Readings	Percent of Total	Observed Maxima Directions () = maxima less than 12%		
All fractures	2419	100	N15W	(N20E)	N82E
6" spacing	687	28	N17W	(N22E)	N83E
1' spacing	957	40	N15W	(N23E)	N80E
1' spacing	1462	61	N16W		N83E
3' to 20' spacing	527	22	(N70E) N15W		N76W
Miscellaneous	332	14	N8W	(N23E)	N85W

Regional Data Summary

There was not a perfect relationship between persistence and intensity. Overall, however, non-pervasive fractures tended to have the smaller spacings, while semi-pervasive and pervasive fractures were more widely spaced. Table V shows a summary of the total data for the eleven quarries. There are two sets in every quarry, with usually greater than 12% maximum, which are orthogonal. The minor sets are not seen at each quarry but they are also possibly orthogonal. Stearns (14) has also observed situations where fracture sets that are apparently orthogonal regionally were rarely observed together at individual data sites.

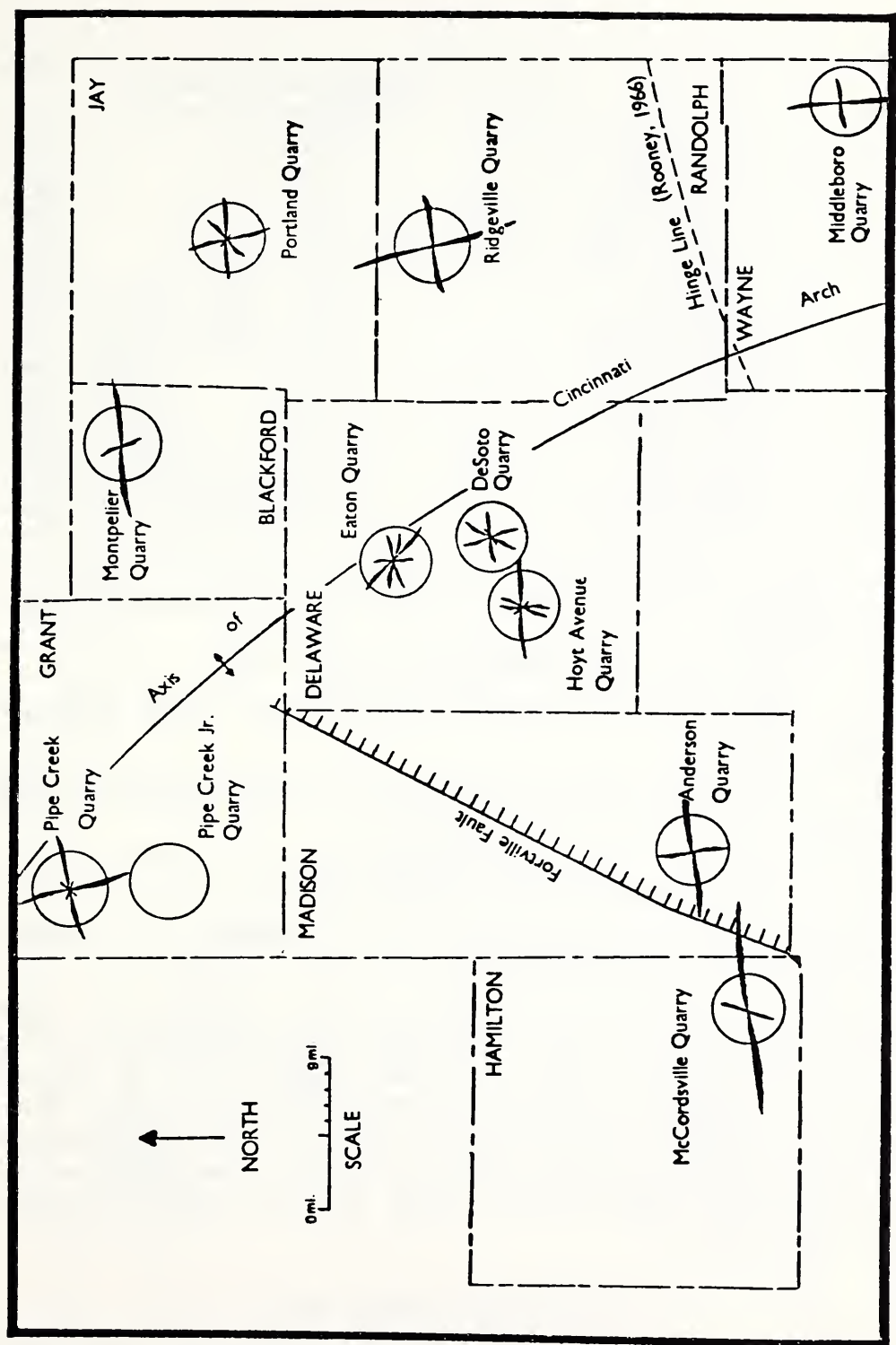


FIGURE 5. Map of study area showing fractures rosettes for pervasive fractures. Circle represents 20% of the total sample for each quarry.

TABLE V *Summary of Fracture Sets for Each Quarry*

Age	Quarry Location	Observed Maxima Directions			
		() = maxima of less than 12%			
Dev.	Anderson Quarry	N6W		N85E	
	Pipe Creek Quarry	N16W		N76E	
Upper Sil.	McCordsville Quarry	(N78W)	(N47W)	(N6W)	N85E
	Pipe Creek Jr. Quarry	(N42W)	(N16W)	(N25E)	(N76E)
Mid. Sil.	DeSoto Quarry	N17W		N25E	(N52E) (N68E)
	Hoyt Avenue Quarry	(N17W)		(N22E)	(N50E) N85E
	Eaton Quarry	(N75W)	(N11W)	(N45E)	N76E
	Montpelier Quarry	N16W		N80E	
Lower Sil.	Portland Quarry	(N74W)	N16W	(N47E)	N85E
	Ridgeville Quarry	N16W		(N22E)	N85E
	Middleboro Quarry	N15W		(N15E)	N76E

The following conclusions are reached regarding pattern consistency across the study area.

1. There is one major orthogonal set (N15W - N82E).
2. There are two minor, possibly orthogonal, sets (N75W - N22E and N45W - N48E).
3. Several quarry faces of different orientation must be examined to see a regionally consistent pattern at individual sites.
4. All fracture lengths (pervasiveness) must be examined to see a regionally consistent pattern at individual sites.
5. All fracture intensities (spacing) must be examined to see a regionally consistent pattern at individual sites.

Further examination of the data involved combining data from neighboring quarries in order to test possible regional differences across the study area (Table VI). There is a subtle suggestion of a 10° regional counterclockwise shift in the pattern progressing from southwest to northeast as seen in grouping of data from the western versus Pipe Creek (northwest) regions respectively (Fig. 6). Grouping of data for Delaware County or for the four eastern quarries displayed similar maxima with more scatter in Delaware County data (Fig. 7).

TABLE VI *Grouped According to Region*

Region	Number of Readings	Observed Maxima Directions			
		() = maxima less than 12%			
Western	400	N5W		N85E	
Pipe Creek	400	N15W		N73E	
Delaware Co.	600	(N15W)	(N22E)	(N46E)	N82E
Eastern	800	N16W		N80E	

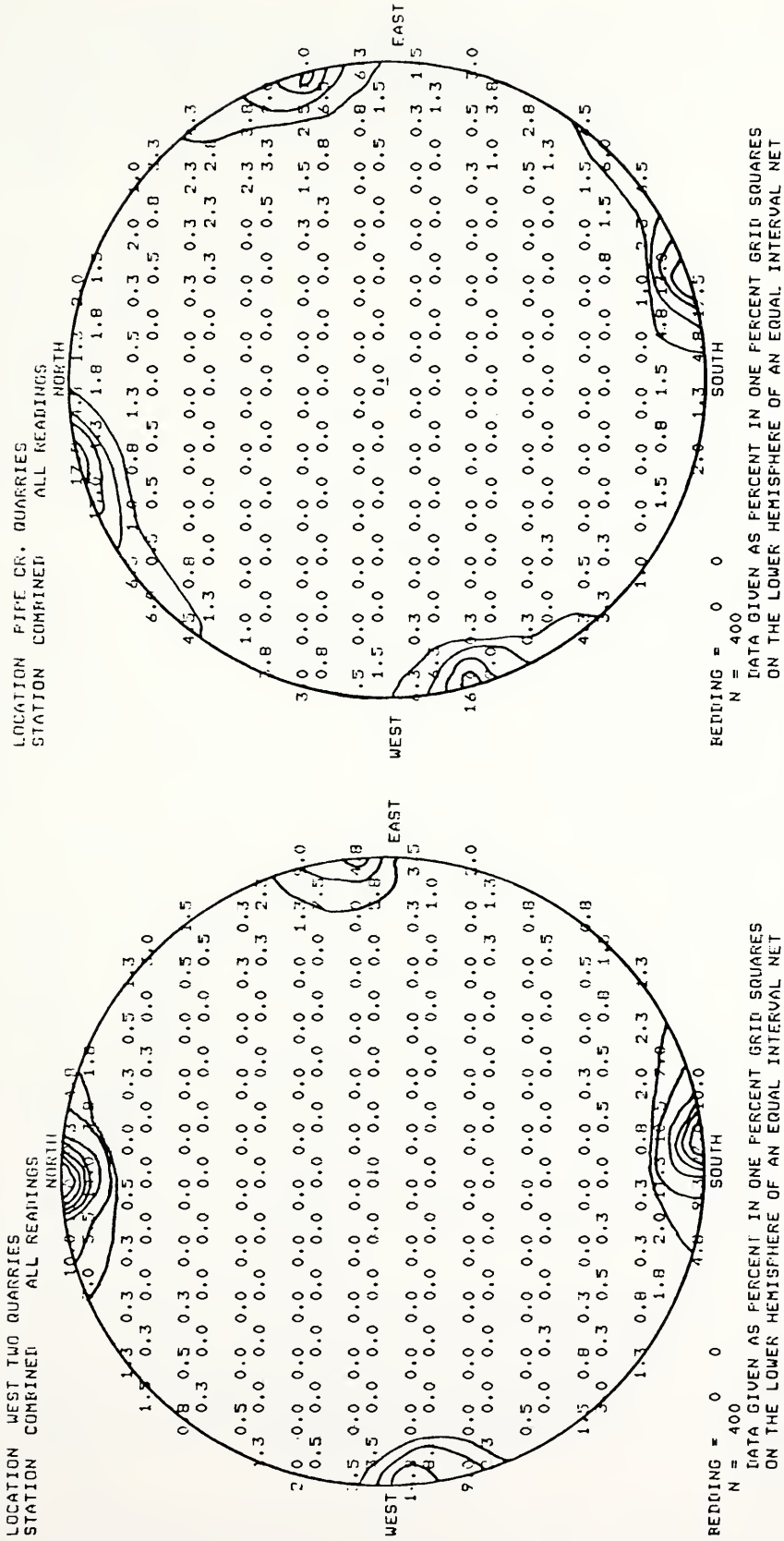


FIGURE 6. Comparison of Western (left) and Pipe Creek (right) regional equal-area net plots.

Quarries were also grouped according to formation age to test for differences in the orientation pattern (Table VII). The pattern is very similar to the preceding regional grouping (Table VI) since in general the formations were found in specific regions. Thus, it would be difficult to distinguish whether the subtle regional differences are due to age, lithology, or geographic position.

TABLE VII *Group According to Formation Age*

Formation	Number of Readings	Observed Maxima Directions () = maxima of less than 12%			
Jeffersonville Fm. (Anderson)	200	N6W			N85E
Wabash Fm.	600	N15W		(N50E)	N76E
Louisville Fm.	797	N15W	(N23E)		N81E
Salamonie Fm.	600	N17W	(N18E)	(N47E)	N81E

Regional Joint Mechanisms

Fracturing may be caused by many factors. Regional fracture patterns have been determined in many places, but their origins are often not satisfactorily explained. It is beyond the scope of this paper to provide an exhaustive summary of alternative explanations. Pentecost (6) has summarized mechanisms of jointing as they may pertain to the study region. While progress is being made, insufficient data exists to provide an acceptable explanation for the generation of regional fracture patterns observed in East-Central Indiana.

The observed regional pattern is apparently very similar to fractures observed in Southwestern Indiana; the dominant joint strikes were slightly west of north and north of east (7). The subtle changes in orientation of the master fracture system suggests a possible relationship to the warping of the Cincinnati Arch (Fig. 5). (One must keep in mind that the Cincinnati Arch is a broad feature and the line denoting the axis of the arch is imperfectly located). The east-west fracture set is dominant in the south-west portion of the study area and tends to strike in a more north-easterly direction in those quarries nearest the axis. The north-south trending set is dominant in the northeast portion of the study area and is to some degree parallel to the suggested axis of the Cincinnati Arch in most of the quarries. If the warping of the Cincinnati Arch is in part responsible for these fracture patterns, additional studies conducted further to the northwest and southeast should reveal fracture patterns having the same approximate relationship to the axis of the arch.

Fracture orientations in East-Central Indiana may be controlled in part by pre-existing fractures in the basement rock. The fault systems in Central Kentucky may be controlled by deep-seated ancient faults in the basement rock (1). The release of residual stresses developed previously in older rock due to compression may also be responsible for fracture patterns in overlying glacial material (3).

The development of positive structural relief with erosional unloading has been cited as the cause for orthogonal fracture systems in the Uncompahgre Uplift (5), near the Bonita Fault in New Mexico (14), and in Southwestern

Indiana (7). The Cincinnati Arch, which trends northwesterly through the study area, was initially uplifted in Late Ordovician time, but renewed uplift occurred during Early-Devonian and Late-Mississippian times (2). This broad anticline was subsequently truncated by erosion producing the present-day pattern of Silurian and Ordovician core with Devonian and Mississippian rocks dipping gently away on either side. A broader Post-Pennsylvanian warping also occurred which regionally uplifted the Cincinnati Arch, the Wisconsin Dome, the Michigan Basin, and the southern part of the Canadian Shield, while the adjoining basins subsided, further adding to the positive relief of the study area (4).

Vertical stress approximately equal to the lithostatic load is responsible for causing initial horizontal compressive stress (12). When positive structural relief and erosional unloading occur, the lessening of the vertical load decreases lateral compression and confinement. Subsequent crustal expansion results in extension fractures and the release of residual stress which may be responsible for regional fracture patterns (9).

The orientation of recent compressive stresses related to the mantle flow pattern that drives sea-floor spreading may be a possible cause of regional fracture patterns. Horizontal compressive stresses striking east-northeast exist in Ohio which might be part of a regional pattern extending to Illinois and Eastern Missouri, where in situ measurements and fault plane solutions of first motion in recent earthquakes indicate similar directions of compressive stress (12).

The secondary fracture sets may also have been brought about by any of the phenomena cited. Additional fracture sets may have been caused by more than one period of compression, uplift, or unloading (14). The lack of uniformity in the amount of unloading, regional compression, and crustal expansion may have also produced any of the secondary sets.

Summary and Conclusions

Three orthogonal fracture systems were found to exist in the Paleozoic bedrock of East-Central Indiana. The master system had fracture sets striking N17W to N6W and N85E to N68E and appeared at every data collection site. The other two fracture systems were less consistent in their appearance and exhibited sets striking N45W and N48E, and N75W and N20E, respectively.

Quarry face orientation was found to be a factor that could bias sampling locally. By examining at least two faces of differing orientation at each quarry this bias was eliminated. It was also determined that the regional patterns were best seen in individual quarries when all categories of fracture persistence and intensity were considered.

Orthogonal tensile fracture systems have been observed in many regions. Typically the secondary regional trends are not observed at each recording station. The mechanism for forming orthogonal systems is still poorly understood but generally their development is attributed to regional uplift and erosional unloading of overburden permitting release of confinement and residual stresses.

The residual stresses could be either recently developed from east to west plate tectonic activity (some have suggested glacial loading) or they could be older. Warping of the Cincinnati Arch would leave some residual stress or perhaps the stresses are imposed from below and are due to stress history in the Precambrian crust.

Acknowledgements

We extend thanks to Drs. Donald Carr and Robert Blakely, geologists, Indiana State Geological Survey, for their aid in procuring information that aided this investigation. The cooperation of the quarry operators who allowed us the use of their facilities to collect data is appreciated. This investigation could not have been completed without it. Mr. George Viconovic offered field assistance to Dave Pentecost who collected all field data presented here. We thank Dr. Harlan Roepke, Dr. Henry Kane, and Walter Pierce for review of early versions of this paper and their many helpful suggestions. Mrs. Jan Vice patiently typed this manuscript.

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Notable Contributions of Early Indiana Geologists

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David Dale Owen was not the first geologist to visit Indiana, nor the first to live in Indiana, but he was probably the first to whom the name Indiana geologist can be applied accurately, and his contributions were major, in considerable part because his observations were applied to virgin territory. The pre-Owen knowledge, or lack of it, is revealed by William Maclure's map of 1818 (6) on which all the bedrock west of the Appalachians was shown as Secondary, equating in modern terms to middle-to-late Paleozoic.

In 1837 the State Legislature authorized the designation of a geologist for the State of Indiana, and Owen was appointed by the Governor. During that year and the succeeding one, Owen made horseback traverses that resulted in his assignment of the bedrock units to the Paleozoic systems already established in Great Britain. All the systemic boundaries remain where Owen placed them. He also correctly separated the systems that later became in America the Mississippian and Pennsylvanian, and he distinguished between the present-day Ordovician System and the Silurian System, even though his work preceded the Sedgwick-Murchison controversy, and the Ordovician System was not proposed by Lapworth until 1879 (4). To accompany his reports Owen prepared, in 1838, an outline map of the geology of Indiana that was never published but was deposited in the State Library, from which it must have been lost or taken shortly afterward, as I am aware of no reference to it except Owen's own (8), in which he described the map in sufficient detail to establish the fact that the boundaries shown must have been essentially the same as those on a map printed in 1846 as a part of an Owen paper published in England (9).

Owen's brief work of the 1837-39 period was followed by a hiatus of 20 years in State-supported geologic activities, except for a few studies by Ryland Brown, Geologic Agent for the Indiana Department of Agriculture. A second episode of activity by David Dale Owen began in 1859, but it concluded soon afterward with his death in 1860, appointment of his brother Richard to succeed him briefly, and then another suspension of any State-supported program in geology.

The geologic effort that succeeded the Owen surveys was established in 1869 as an organization named the Geological Survey of Indiana. Unlike the two earlier episodes of activity, the Survey was a unit of State government, whereas the Owens had been appointed as individuals to fulfill specific assignments. The new Survey was headed by Edward Travers Cox, an assistant of David Dale Owen during and in surveys for the United States government of the upper Mississippi Valley and areas within other states. His principal contribution, in my view, was the establishment of a sound and continuing State geology program. The annual reports issued during Cox's 10 years in office covered the mineral resources recognized to that time and offered the first specific

information designed to encourage development and to attract industry to the state.

Next in chronological order, I would point to the work of A. J. Phinney, who in the 1880's was the author of various papers in the annual reports of the organization that by then had come to be called the Indiana Department of Geology and Natural History, but Phinney's most notable achievement in Indiana geology was done under the aegis of the Federal, rather than State, government, and published by the U.S. Geological Survey (10). It was the 160-page paper entitled *The Natural Gas Field of Indiana*—one of the earliest and best in the annals of petroleum geology.

The sixth State Geologist of Indiana, Willis Stanley Blatchley, was, in my judgement, the greatest builder of organizational strength during the period covered by this study. He served from 1895 to 1910—a longer period than any of his predecessors; time in office is surely a factor in establishing a program, but from the beginning of his tenure he demonstrated an unusual ability to identify and attract capable scientists to his organization. The authors of the papers in annual reports issued during the Blatchley years constitute a merit list in geology. To avoid comparison and unintentional ranking, I list a few of them in alphabetical order: George Ashley, one of the first and greatest coal geologists; E. R. Cumings (to be mentioned further in this paper); August Foerste, the sage of the Silurian; T. C. Hopkins, author of valuable reports on the geology of the industrial minerals, and a turn-of-the-century sedimentary petrologist before the term was used; E. M. Kindle, stratigrapher and paleontologist in the Silurian and Devonian rocks, and bibliographer and cataloguer of literature and fossils; and C. E. Siebenthal, expert on building stones and cement, and the geology of the rock units used for both.

To shift now from bedrock to unconsolidated deposits and the processes that formed them, we can hardly regard Frank Leverett as an Indiana geologist, but surely he spent much time in Indiana doing geologic work for the United States Geological Survey, and it was in Indiana and other Midwestern states that he conducted much of the field work leading to early precepts of continental glaciation and distribution of glacial materials. His works and maps were published, beginning before 1900, in annual reports and monographs of the U.S. Geological Survey, and for Monograph 53 (5) his co-worker and co-author was Frank B. Taylor, who was a native of Fort Wayne. The Leverett and Taylor manner of depicting glacial geology was notable in lending pattern and reason to the materials and the events responsible for their distribution.

Frank Taylor has received less recognition than might have been expected for proposing, in 1910 (12), some two years before Alfred Wegener, reconsideration of continental drift, which had been suggested much earlier by Francis Bacon in 1620 and Antonio Snider in 1855 without receiving serious attention. Both Taylor and Wegener were impressed by the jigsaw fit but had problems in explaining the mechanism. Wegener became noted for his advocacy and Taylor did not, in part because he lacked a DuToit. Doctor Johnson, it may be argued, would probably have been well recorded in history without Boswell, but Wegener, equally probably, would not have without DuToit, just as Hutton's views might have had much less impact but for Playfair.

A turn from glacial geology to geomorphology and physiography is not abrupt. Clyde Arnett Malott, who spent a productive life studying the geology of Indiana, made his own contributions to knowledge of the State's glacial geology, and he was a sound stratigrapher, as evidenced by his work with Meramecian and Chesterian rocks, among others, but I would place his unique accomplishments in two other fields—karst processes and geomorphology, and descriptive physiography. In the former field, he utilized the Mississippian karst terrane of southern Indiana and some other Midwestern regions to formulate concepts of underground drainage and its development in numerous, generally short, papers published principally in the Proceedings of this Academy. His other unusual achievement to which I refer was to name and describe (7) seven bedrock physiographic provinces that cover all of southern Indiana south of the Wisconsin glacial boundary and extend, recognizable from subsurface records, beneath the thickening glacial drift to the north. Doctor Malott preferred to term himself a physiographer rather than a geomorphologist, and I believe that he was correct in doing so. He had the unusual ability to describe terrane in a manner that made it recognizable to persons seeing it for the first time. In the half-century since Malott named the physiographic units, no changes have been made in their designation, possibly because each unit is as Malott described it. I cannot be objective about Malott's accomplishments, as it was his enthusiasm for geologic work and his ability to transmit that enthusiasm that drew me into the field. I am not alone.

Approaching now perilously close to the 50-year-ago limit that I have set arbitrarily for this review, I call your attention to the fundamental works on reefs and their environment by E. R. Cumings, long-term faculty member and chairman in the Department of Geology at Indiana University, and R. R. Shrock, an undergraduate and graduate student in that department in the 1920's. Doctor Cumings was well-known long before the reef work was undertaken for his research in paleontology and stratigraphy, particularly of Ordovician and Silurian rocks. Doctor Shrock went on to other triumphs as faculty member at the University of Wisconsin, where he was co-author with William H. Twenhofel of the text *INVERTEBRATE PALEONTOLOGY* (13) on which many of us were reared. Later he was co-author with H. W. Shimer of the revised *INDEX FOSSILS OF NORTH AMERICA* (11) and became a faculty member and ultimately departmental chairman at the Massachusetts Institute of Technology, but these were achievements that post-dated the period covered by our review. The work on reefs, beginning with a paper in 1927 in the Proceedings of this Academy (1), followed in 1928 by Publication 75 of the Indiana Geological Survey (2), and another with greater geographic coverage in the same year in the Bulletin of the Geological Society of America (3), have joined the ranks of classics. Reef geology, largely neglected since Darwin's day, was principally of academic interest at the time of the Cumings and Shrock studies, but there are few subjects of greater significance in petroleum geology today.

Numerous efforts of the past 50 years may merit inclusion in a list of notable contributions when they come of age and can be evaluated in the light of history.

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Regional Land Use Planning in Jasper, Newton, Pulaski, and Starke Counties, Indiana¹

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Introduction

Jasper, Newton, Pulaski, and Starke Counties are located in northwest Indiana, the second tier of counties south of Lake Michigan. The population of this area in 1970 was 63,849 and by 1975 was estimated to have increased by more than 15% or nearly 10,000 persons. As a response to future projected growth trends, the Kankakee-Iroquois Regional Planning Commission (K-IRPC) began to develop a plan in 1975 which was completed in 1978. The land use Growth Policy Plan (4) provides a regional development framework for future urban growth decision making. The work "framework" represents the philosophy of the Plan in that it illustrates a broad superstructure within which individual local governments retain a wide latitude to plan for local needs. The recommendations of the Plan provide an indication of the most desirable general location for new urban growth based upon environmental, social, and economic information as well as goals and policies developed by a citizen advisory committee.

Methodology

Land Use Inventory

Growth management planning must consider the existing land use conditions since further growth in the region should be in harmony with development that has already occurred. Therefore, in 1975 and 1976, a detailed land use inventory was conducted to determine the existing uses of the land. All land within the four counties was classified according to a modified Level II system (1). Aerial photographs at a scale of 1 inch equals 2,000 feet served as the primary basis of the inventory. Field survey, topographic maps, plat books, and contact with area residents were other sources of information used to determine land uses. The resultant map is at a scale of 1:62,500. A statistical summary was also prepared which described acreage in each category of use employed in the inventory (Table I).

Environmental Inventory

An environmental inventory was prepared to determine the capability of the land to sustain and support urban development while minimizing resource loss. Eight different factors drawn from the categories of soils, hydrology,

¹This project was financed in part by a grant from the Department of Housing and Urban Development under provisions of Section 701 of the Housing and Urban Development Act of 1954, as amended.

TABLE I *Land Use Inventory by County (1975)**

	Jasper	Newton	Pulaski	Starke
Residential (Incorporated)	1,554	883	665	820
Commercial & Services	617	659	317	238
Industrial	1,063	172	121	179
Transportation, Communications	3,333	1,826	957	1,452
Utilities				
Institutional	370	72	386	313
Strip & Clustered	3,684	3,331	869	4,233
Residential				
Open & Other	1,581	588	364	1,681
Cropland & Pasture	304,043	220,623	238,436	148,854
Deciduous Forest	37,988	28,341	33,722	34,034
Evergreen Forest	762	1,267	262	2,592
Streams & Waterways	1,033	210	659	413
Lakes	214	1,794	99	1,841
Non-Forested Wetlands	2,547	4,410	147	3,418
Strip Mines, Quarries	827	144	116	60
Totals	359,616	264,320	277,120	200,128

*Acres

geology, flora, and fauna were mapped using a transparent overlay technique. Three limitation classes were developed using shades of gray to depict the general potential of an area for urban development. A slight rating has few or no limitations for growth and is shown clear. A moderate rating indicates a level of limitation that reduces the desirability of an area and is shown in light gray. Some corrective measures are required in these areas. Severe ratings, depicted by a darker gray indicate unfavorable conditions which greatly restrict urban uses. Special engineering, design, and resource loss potential are problems which need to be overcome. The limitation system approach is useful in that it does not eliminate an area from urban development but instead indicates the degree of environmental modification needed. The maps were prepared at a standardized scale of 1:250,000 and can be viewed singly or in combination. The overlays prepared are not intended to be site specific but rather offer a general perspective on the potential of an area.

Three separate soils map overlays were prepared. Soil association data (2, 6, 7, 8, 9), were evaluated and classified into severe, moderate and slight classes. The general soils evaluation for urban development map depicted properties such as wetness, shrinking and swelling, poor bearing capacity, and high slope. Properties of the septic tank absorption capacity map were water moving too slowly through soil, wetness, and soil flood. Finally, prime agricultural land was classified utilizing the average productivity index, (6, 7, 8, 9) and a stratification of the resultant values.

Hydrologic factors considered for separate maps were 100 year flood hazard and groundwater availability. Information drawn from the U.S.G.S. indicating flood prone areas was mapped on lands adjacent to the four major rivers of the region as a severe limitation. These include the Kankakee, Yellow, Iroquois, and Tippecanoe Rivers. Groundwater availability was classified with

regard to the gallons per minute (gpm) supplied and was derived from data supplied from Kankakee River Basin, Indiana and Wabash River Basin Comprehensive Study (5, 10). Less than 100 gpm was classified as a severe limitation, 100 to 500 gpm as a moderate limitation, greater than 500 gpm as slight limitation for urban development.

A geologic map overlay was prepared. The properties classified into severe, moderate and slight limitations included poor bearing capacity, slumping soils, poor slope stability, high susceptibility to frost damage, and permeability. Geologic judgements were supplied through the Indiana Geological Survey (3).

Flora and fauna factors considered in separate maps were forested areas, and wildlife population and stream fisheries habitats. Information obtained from Arrow Head Country Resource Conservation and Development (R. C. & D.) (2) depicted the general location of forest lands in tracts greater than 60 acres and were classified as a severe limitation to development. Wildlife population and stream fisheries habitats data were also obtained from Arrow Head Country R. C. & D. (2). Good to excellent habitats were classified a severe limitation to urban growth.

The assessment of the above environmental inventory overlays yields the urban composite map (Fig. 1). This was achieved by viewing the eight environmental overlay maps in combination on a light table with the darkest areas indicating the environmentally most sensitive location for urban development. In like manner, the lighter areas represented the most "ideally" suited areas for urban development. The urban composite map represents a simplification of the various shades of gray and is classified into areas of severe, moderate, or slight environmental limitations for urban development.

Social and Economic Growth Potential Factors

To obtain a measure on the potential for future urban development in the various jurisdictions within the four counties, a weighted rating system addressing a number of selected socio-economic elements was prepared in consultation and with the unanimous approval of the Four County Growth Policy Committee, a citizen's advisory committee. Each of the sixty governmental units (townships, towns, and cities) were assessed for the presence or absence of selected socio-economic elements. Numerical point awards with values ranging from one to ten were assigned for a jurisdiction's commuter potential, major highway access, existing or planned water and sewer utilities, historical population growth rate values for three time periods (1930-1970, 1960-1970, 1970-1975), airport, railroad, or industrial park. The total point values for each of the governmental jurisdictions were compared with each other and stratified into high, medium, and low potential growth ranges. Points award ranged from a high of 50 points to a low of 9 points. A map was prepared to summarize the results (4).

Land Needed for Urban Conversion

Land use needs estimates were calculated to indicate the minimum amounts of rural land which will be converted to more intensive use by urban growth. The needs estimates, in acres, are based upon population projections, the 1975 land use inventory, local zoning standards, and the numbers of persons per

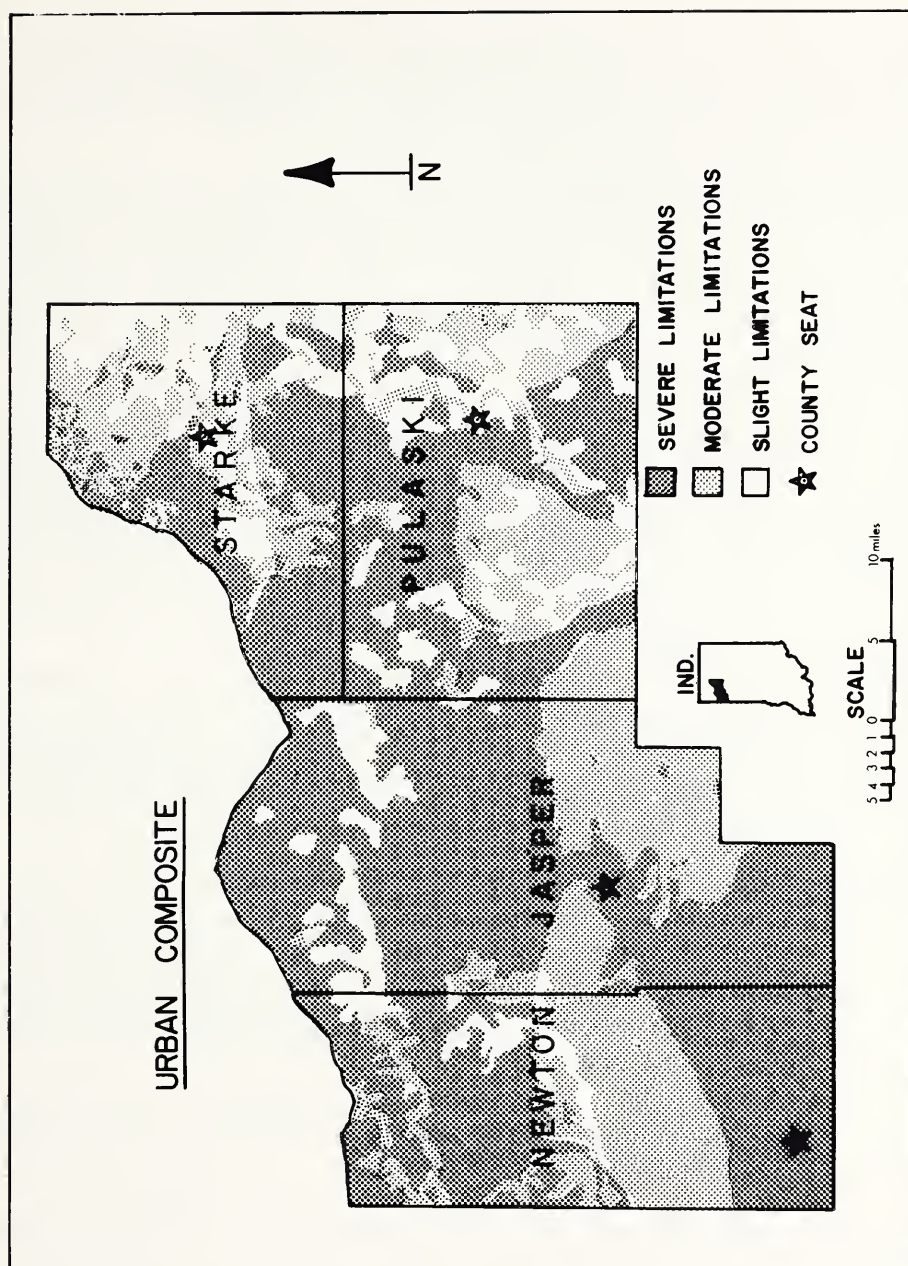


FIGURE 1. Map of Area

household. The resulting urban land use needs estimates for each of the governmental units were compared with each other and stratified into high, medium, and low ranges. A map was prepared to summarize the results (4).

Local Plans and Ordinances Inventory

Existing plans and ordinances have been inventoried and incorporated into the planning process. Special emphasis was placed upon the current zoning in the unincorporated areas. A zoning map of the unincorporated areas was prepared at a scale of 1:250,000 which delineated residential, commercial, and industrial areas.

Areas of Critical Concern Inventory

During the fourth quarter of 1975, specific critical concern areas were polled from individuals and organizations within the four county region. Ideas and problems identified were compiled (4) and mapped at a scale of 1:62,500. The four major areas defined included natural, environmental, cultural, and health, safety and convenience.

Current Growth Directions

Current growth directions and growth centers were mapped by comparing highway photographs, topographic maps, and aerial photographs within the time frame 1950-1975. Clusters of new urban development were identified. The resulting map at a scale of 1:250,000, portrays by location and compass direction strong growth centers of urban development since the 1950's (4).

Goals and Policies

Simply stated, goals and policies are statements that provide a framework for decision making. To varying degrees, they describe long range community desires and courses of action that can guide future development within the four county region. The Four County Growth Policy Committee developed and unanimously adopted the following goals:

1. To promote orderly growth that compliments and enhances established communities, the physical environment and preserves the rural character of the four counties.
2. To promote the conservation and improvement of the air, land and water resources of the four counties.
3. To promote the conservation and best use of our man-made and natural resources.
4. To promote energy efficient development patterns.
5. To promote resource management awareness among the area's citizens.
6. To preserve the traditional concept of home rule and local control over zoning ordinances.
7. To promote the collection of additional detailed environmental and socio-economic information for use in local growth related decision making.
8. To encourage urban growth which would be compatible with the agricultural community.

Conclusion

The fundamental purpose of regional land use planning is to provide technical assistance and guidance to the elected officials, local plan commissions, and citizens in the sound management of land resources for urban growth. The planning process provides a development framework within which today's public and private growth related decisions can be made with some sense of the future.

Preferred urban growth areas recommendations were prepared for the year 2000 based upon the planning process outlined above. Retaining a regional perspective, the recommendations indicated a general location for new urban growth which was usually expressed as a compass direction adjacent to an existing incorporated community. During the early months of 1978, the planning process was summarized and presented to the units of government within the region. Seventy-five percent of the local governments endorsed that portion of the Plan relating to their jurisdiction. Currently the Kankakee-Iroquois Regional Planning Commission is seeking to upgrade the efforts of the local units of government in the implementation of the Plan.

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Opposition to Reservoir Construction

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Introduction

For decades the American public apparently assumed that those in charge of reservoir construction were well informed, intellectually honest and were constructing the man-made lakes for the improvement of society. In recent years however especially since World War II ended, it seems to be increasingly clear that some promoters were influenced by their own financial interest and the betterment of their allied associates. Now years and decades after the construction of some reservoirs, evidence has accumulated to indicate that too many of these have been built in the wrong places at the wrong times for the wrong purposes. Construction was too often poor and sometimes maintenance careless or almost non-existent.

Some conservations and environmentalists are stressing that reservoir sites are as subject to exploitation and misuse as forests, soils and other natural resources. The public is becoming increasingly aware that proposed reservoir construction should be questioned and that both economic and environmental impact statements and assessments should be prepared before finalizing the decisions to build reservoirs.

Opposition to Construction

Multiple use. The term "multiple use" is about as ambiguous as the term "miscellaneous use". Consequently it is often intentionally or unintentionally misused to confuse. Multiple use is indefinite unless the user of the term designates primary, secondary and perhaps tertiary uses. Too many promoters use the term in such a loose way that it reminds one of the tent salesman's medicine claim that the bottle of stuff would cure all ailments from toeache to headache and all pains in-between. Promoters seeking general public support often tell anyone who asks about a certain use that the reservoir is for the inquirer's desired use whether the uses are as incompatible as irrigation and flood control.

At no time during the promotion for the construction of the largest man-made lake in Indiana, Lake Monroe, were the primary and secondary uses of the potential reservoir identified by local newspapers although the state did publish Report of Investigation Monroe Reservoir Salt Creek near Harrodsburg, Indiana For Flood Control, Increasing Low Flow and Allied Purposes (1). One may notice that neither recreation or water supply are listed as primary uses in preceding title, but are assigned to the catch-all term, "allied purposes". But today some people still try to claim that recreation and domestic water supply are primary uses. During the time the Monroe Reservoir was under construction some farmers and students believed and argued that the reservoir would provide

water for irrigation until they were challenged to locate the outlet from the reservoir through which the irrigation water would flow.

False information. There seems to be a natural tendency for many promoters of reservoirs to exaggerate and for the media to repeat the exaggerations without adequately checking to learn the facts. It was said and repeatedly announced that the Monroe Reservoir was to create a 20,000 acre lake and that it was to protect local farmers from flooding. Even a local man representing a state agency made the false claim about its size. After the "balleyhooing" was over and the lake put into operation for one of the two primary purposes of flood control, the water level is now maintained near the 538 foot elevation above sea level. When the water stands at the 538 foot level Lake Monroe covers 10,750 acres (2).

As for local flood control in the Salt Creek watershed, 7,800 acres of cultivated land in the Salt Creek valley below the dam receives flood protection. But 10,750 acres are permanently flooded by Lake Monroe. Furthermore an additional 7,700 acres were taken out of farm production because the lake might reach spillway level (556 feet above sea level) when water storage needs become critical.

The public by sad experience has correctly learned to be wary of promoter's claims. Reservoir promoter statements should always be checked and double checked because far too many reservoir promoter's creditability is low.

Loss of cultivated land. Most reservoirs are built in valleys by placing dams across creeks and rivers. Often, if not usually, the richest soil is on the valley floors and flood plains. The farm owners who live on the land view it as a place to earn a living and as a home. They and their ancestors may have resided in the area for decades if not a century. In contrast, some urban people may view a valley as a good reservoir site and believe the construction of a reservoir will improve their economic income and raise their standard of living. Unless an economic impact statement is prepared, the importance of farming in a valley is underrated especially in relation to the local governmental units such as townships and counties. Monroe County has a low rating as an agricultural county because too much of the surface is covered with hills, ridges and slopes with little if any top soil. But reservoirs are not built on hills and ridges; the smaller the amount of good farmland in a township or county, the more critical the loss of the better soils for farming becomes to the smaller governmental units.

Unearned increments. Undoubtedly there is a large residue of bad feeling which lingers on for decades after a reservoir has been constructed because people who lost their farm and homes—some due to fear of having their land submerged and others forcefully by eminent domain. In addition sharpie land buying practices have galled many landowners, their relatives and friends. Many farmowners live comparatively isolated lives and do not have as ready accessibility to valuable information as do land speculators. The latter upon receiving inside information as to whether a reservoir is to be built or not and where, comb the countryside to get control of huge acreages at relatively low prices. Later the displaced farmers see their former land holdings being sold for

five, ten, twenty or a hundred times more than they received. The speculators have contributed nothing to the value of the land. And if the reservoir is being built with tax money, the tax payers have also been rooked.

A high percent of reservoir construction costs could be paid for if governments would take additional land adjacent to the land where the water is to be stored at the same price as that of the reservoir storage land, and retain profits from rising land values and sales due to the construction of the lake. There is no justification for constructing a reservoir with tax dollars unless it is to benefit society. The benefits should result from both making water available and gaining the increment from the raising price of land within a fifth, or quarter or half a mile of the reservoir depending on its size.

Helping pay for the cost of building public reservoirs from rising land values is not a new idea, but one which is not practiced in most states (because it would benefit the taxpayers rather than the speculators). This type of management was put into operation nearly 50 years ago in the 1930's by the Tennessee Valley Authority. Also this type of management was demonstrated over a decade ago by a privately-operated Beech River Development Authority which constructed a series of dams on the Beech River located roughly midway between Nashville and Memphis, Tennessee (3).

It is surprising how uninformed or misinformed some otherwise well informed people are about the distance the Army Corp of Engineers may acquire in fee land around the reservoirs they build with alert, dedicated state and local leaders and with community support. The minimum to be acquired is 300 feet horizontal freeboard allowance above the top pool elevation for storing water for flood control, navigation, power, irrigation and other purposes. In addition to requiring land for the full control pool, the Corps is authorized to acquire lands for fish and wildlife, for recreation, for operation and maintenance, and then the 300 foot strip (4). Planners for the reservoirs have considerable latitude in taking more land which is relatively easy to justify if the taking is desired by state officials and if local opposition is slight. But speculators can be very alert, vocal and influential. Too often the farmers and tax payers are not happy and become leary of reservoir construction and promoters.

Quick profits. Why is reservoir construction called an economic stimulant or generator? Who quickly gains from the construction of a public reservoir by money being pumped into the community? There are relatively quick returns to land speculators, those engaged in transfer of land titles such as lawyers and real estate recorders, bankers, construction contractors, equipment and material suppliers, transporters and construction employees. Often the community "movers and doers" are engaged openly or behind-the-scenes in these economic activities. The income from reservoir construction for these persons (perhaps less than one percent of the population) will far exceed what they will pay in taxes, therefore construction will greatly benefit them even if the construction does not have a benefit cost-ratio of 25 cents benefit on a dollar. Some of those who will gain quick lucrative benefits, attempt to divert attention from their windfalls by promoting the idea that the reservoirs are being built by tax dollars

brought home from Washington D.C. which would otherwise be wasted on something else.

If for no other reason economic impact statements and scientific truthful cost-benefit ratios need be prepared and subject to public scrutiny and discussion in order to hold the "fast buck" element in society in check.

Cost-benefit ratios. The creditability of at least two U.S. governmental agencies that build most of the federally-constructed reservoirs is so low (whether justified or not) that the public has begun to distrust the term cost-benefit ratio. Statistics can be rigged to indicate less than actual costs and drastically overrate unrealistic benefits. Overruns in project costs once construction is underway are often enormous thus making a farce out of cost-benefit ratios.

The intellectual honesty of proponents of reservoir construction and the cost-benefit ratios they prepare are often rightfully questioned. Some people believe that some governmental agencies will prepare cost-benefit ratios to indicate favorably whatever the greatest pressure group wants. If a state's two U.S. Senators and enough of the state's leaders and the public (as indicated by the media) are lobbying for a reservoir construction, statistics can be provided, for example, to build a 9-foot channel in the Wabash River. When one of the two former Indiana senators is defeated by an opponent who is opposed to the reservoirs and waterway construction, the cost-benefit ratio can be refigured and the cost-benefit ratio can show that the reservoirs should not be built. But don't be too sure that the first batch of statistics will be discarded. They may well be carefully filed to be revised later to indicate that the project is again beneficial when the political situation changes. There are numerous examples of overestimating the benefits and under estimating the costs. Enormous overruns over budgets for reservoir construction have been a continuous, monotonous disgrace. Then, often attempts have been made to make inflation the scapegoat. Too often a too low an inflation rate had been considered in the cost-benefit ratio computation, although inflation is nothing new. Cost-benefit ratios unless evaluated by an independent honest private agency cannot be trusted and are too often a smoke screen.

Ensuing problems. Where a public reservoir is to be constructed fully or partially with federal tax dollars, some people (if not most) at first may be made to think that reservoir construction is something of a Christmas gift. But often after reservoirs are built reality suddenly strikes like a thunderbolt. There the reservoir sits without adequate roads for people to get to and from it. Developers rush in and start construction of houses and commercial buildings where there are inadequate building codes and no available running water, sewers or solid waste disposal. Population shifts disturb the school systems, the sheriff's program and other county public services. Too often there is economic disruption bordering on chaos. Costs soar and tempers flare. Some people wish the reservoir had never been built, or if it had, in some other county.

Failure to plan and zone. Economic and social disruption could have been held to a minimum if the county or counties in which the reservoir is to be

located had area or county planning and zoning agencies, and if the administrators of these agencies had anticipated and cooperated in problem solving. However, some if not many reservoirs are built in counties that do not have county or area planning. Or in other counties planning and zoning exists in name only and have no professional planners and/or only small inadequate staffs with meagre budgets. It is difficult enough to get cooperation within a county, but to secure intercounty cooperation is more difficult. Official intercounty regional planning does not exist throughout all states. Too often the need for intercounty planning is not understood or supported (5). As a result, often reservoirs are constructed before post World War II soil surveys, water, sewage, roads, schools and other surveys are made. So often economic impact studies have not been made to justify the project.

Upstream-downstream benefits. Too often the upstream-downstream benefits of a potential reservoir are not presented to the public before the decision to build it is reached. To protect some people's land in the floodplain below the dam from flooding, it is necessary to flood permanently other people's land above the dam. People upstream from the dam usually have better access to the reservoir's water and also greater opportunities to sell land at a profit for non-agricultural purposes than do the downstream landowners. However, the latter have a better location if the reservoir water is destined primarily for irrigation. These two types of benefits, upstream and downstream should be clearly discussed before construction, but too often are neglected. Consequently, dissention develops later during construction and filling of the reservoir or after the reservoir is filled.

Construction destruction. Beauty is in the eye and mind of the beholder. Many urban people see no beauty in continuous stretches of corn or soybean fields in the U.S. corn belt. Nor did many pioneers see the beauty of vast stretches of prairie which spread from central Ohio to eastern Nebraska where the beans and corn now grow. Some people do not want the habitat of nature's population of fauna and flora destroyed. Other persons could not care less. Those promoting reservoirs see beauty in bulldozers and other twentieth century machinery that tear up trees and forests, move across fields gouging and destroying soils, piling up rock and earth and pouring enormous piles of concrete to construct reservoirs. They find pleasure in visualizing the reservoir's waters being used for recreation, irrigation, navigation, domestic and industrial water supplies, flood control, electricity, restoration of wildlife and other uses. Consequently, there is a conflict in the destruction and the creation of beauty.

Lake deterioration. Perhaps two decades should pass before attempts are made to judge whether the after construction beauty exceeds that of pre-construction. Unfortunately too often in a few years it becomes obvious that the public is misusing and abusing the man-made lake. Some evidence of rapid deterioration are 1. excessively littered and cluttered lake shorelines and adjacent areas, 2. excessive growth of weeds encouraged by silting and pollution, 3. badly eroded shorelines and 4. the rapid aging of the lake by the eutrophication.

Cost-benefit ratios become a farce if a reservoir's projected life is cut in half or more by poor management and abuse use. Both small and relatively large lakes constructed with city, state and public funds have had their storage capacity cut from 50 to meanly 100 percent in a few decades.

Where stormwater runoff carries large quantities of silt and minerals in the form of human wastes, industrial wastes and from agricultural operations, these materials settle at the bottom of reservoirs creating an ideal growing habitat for water weeds. Most of the reservoirs and natural lakes in the eastern United States are having their viability seriously threatened by a vascular aquatic plant known as *Myriophyllum Spicatum* (6). The plant is known for its explosive local growth and its regional spreading rate. It started from a single beachhead on Chesapeake Bay only 15 years ago.

Inadequat dams. The inadequacy of many dams as to site, design, materials used, construction, maintenance and other factors are well recorded. But each disaster involves a limited area and the impact is not great in other regions. Natural floods are so common that a man-made flood due to bursting of a dam is just another flood. But these disasters have happened so often in so many different geographic regions that more and more people are concerned. Consequently when the huge Teton dam in the West crumbled in 1976 and soon thereafter the Toccoa Dam collapsed killing 38 people of a small northeast Georgia Bible College in November 1977, a storm of criticism arose, questions were asked and a shocking situation revealed. In 1972 Congress approved an inspection program aimed at preventing such disasters as the Teton and Toccoa dams but as of November 7, 1977 not one dam had been inspected (7). Loche Mouton, a spokesman for the Army Corps of Engineers, attributes this failure to a lack of funds and explains that Congress authorized just enough mney to make an inventory of the nation's dams. David Conrad of the American Rivers Conservation Council believes "Congress is more interested in getting new dams built than it is in the safety of dams already constructed." After the Teton disaster the Bureau of Reclamation reviewed all its 330 dams and found several to be endangered. The Army Engineers inspects the approximately 400 dams it built "all the time." However, a civil engineering professor in 1977 told Congress that at least 14,000 United States dams have been built without control over their design or construction. The Coalition for Water Project Review (which includes 21 environmental groups) have opposed the construction of some dams as has President Jimmy Carter but Congress apparently is not influenced. In 1977 Congress appropriated more than \$3 billion for federal development agencies, but just \$15 million for their safety.

Some groups concerned with dam safety maintain that dam construction should stop until the safety questions are answered and such measures implemented. According to Sport Fishing Institute there are about 1,500 reservoirs larger than 500 surface acres in area existing in the United States in 1978 (8).

Restricted dam use. Apparently dams are not always designed so that the public will gain the greatest benefits. It is difficult for citizens in the Ohio River

watershed and some throughout the country to understand the failure to install electrical generating equipment in many of the permanent dams now in operation and some under construction across the Ohio River. It is still an engineering mystery as to how it was economically feasible to build a special structure adjacent to the Markland dam near Vevay, Indiana to generate hydroelectric power, but not feasible to place the penstocks in Markland dam when it was constructed. Some persons believe that penstocks were not placed in the Ohio River dams because the Eisenhower Administration was opposed to federally-developed waterpower. The Eisenhower Administration gave the privately-owned company the right to build the additional structure to generate electricity after the Markland dam construction started and after John F. Kennedy was elected president.

Fortunately the Markland dam was so constructed that it formed the foundation for a potential bridge. Years later in 1978 a bridge was completed and opened to traffic. Why have not more dams across the Ohio River been designed so as to provide the foundation for a bridge when the dam is being built or so a bridge could be added later?

Cesspool reservoirs. Following World War II the tempo of developers building housing areas around large ponds or reservoirs increased. Small intermittent or permanent streams were dammed and the houses cited in the adjoining wooded areas. Sometimes the man-made lake was to provide domestic water as well as fishing, swimming and boating. A home in the country side with *a view* through a picture window and from a porch and patios became the slogan to sell property to those who enjoyed the outdoor scenes and living, and knew little if anything about the physical environment. Too often the soils around the reservoirs were not suitable for septic tank and fields systems, or these were improperly built and used. Often these septic tanks, built to accommodate a limited amount of water from wells, cisterns and ponds, proved too small when the countryside homes were connected to rural waterlines. In about 10 years or less depending on quality of construction and misuse by the owner, the septic tanks start draining into adjacent lakes. The odor from both the septic tanks and the lake often becomes so strong that patio and other outdoor living becomes impossible. Now the owners in addition to having a lake view have a lake smell as the reservoir in reality becomes an uncovered cesspool.

Fleecing. Not only have the property owners seen their house settings deteriorate and their property values decline but they also learn that their tax dollars have been misused or squandered on water projects in another state. For example, environmentalists have criticized a number of federal projects such as: "... a dam built in Colorado at federal taxpayer's expense which benefits only a handful of farmers to the tune of \$1 million each" (9).

Other objections. There are other objections to reservoir construction both major and minor such as misrepresenting the reasons for building it or attempting to justify the reason(s) for construction after the dam is started or completed. Country people often object strenuously to the lifestyle of people attracted to reservoir areas for recreation. Regardless of why people have taken

a position against reservoir construction, in recent decades their numbers have increased and so has their influence.

Author's Position

In no way does the author wish to leave the impression that he is against all reservoir construction nor would he attempt to refute so many of the just criticisms of such constructions in the past. The demands for both economic and environmental impact studies are justified and are trends in the right direction regardless of the opposition of the speculators, developers and "fast buck" manipulators. Cost-benefit ratio statistics and statements need to be published, critically evaluated and there should be ample time for the opposition to study and refute, if possible, the studies made by the proponents.

Public hearings should be conducted in such a way that they are not shams and farces. People are too alert today not to recognize deception of public agencies. One of the unfair public hearing gimmicks is to provide proponents with hours of time and then when everyone is physically tired, if not mentally, and need to go home, or some have left the hearing, the opponents are provided only limited time. This practice has been engaged in repeatedly. Often another type of deception is to keep potential opponents from seeing the material, prepared by the proponents with tax dollars, early enough before the public hearings so that they could have carefully examined and possibly refute some of the content and conclusions and/or suggest alternatives or additional alternatives.

There is often ample justification for building a reservoir but the "bulldozing" tactics often used to push through the decisions before the opposition can get a word in has left part of the public dissatisfied and has contributed to the increase of those opposing reservoirs.

Why should not states prepare published surveys of major potential reservoir sites? If such information were available and the sites characterized, it might seem reasonable to pass legislation to protect some of the sites from irretrievable development for some other purposes.

Practicing democracy, intellectual honesty and just business practices, and Christian ethics could eliminate much of the opposition to reservoir construction. There will always be opposition to changes in the use of land and usually a few will always be unconvinced and hurt. However, the changes should be made for the good of society as a whole. The public and the landowners should not be deceived by the few.

But as long as Congress and the public do not support Republican or Democratic presidents (10) in their attempt to curtail expenditures for questionable reclamation projects and the projects of the Army Corp of Engineers in the Public Works Bill there seems to be little hope for federal improvement (11).

Whether the 95th Congressional support of President Carter's 1978 veto of the Public Works Bill will start a trend to a more responsible fiscal attitude or not only the future can reveal.

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Customer Distribution of Selected Terre Haute, Indiana Shopping Centers

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Introduction

Numerous research projects have been undertaken with the purpose of investigating customer distribution and travel behavior. Relationships in distance traveled, desirability of goods, and many other variables, have been taken into consideration. New research into the problems of customer behavior involves a unique combination of independent variables or new methods of obtaining the necessary data. The analysis of data obtained from the method is a complex relationship of many variables and is not of primary concern within the scope of this research.

The Problem

In a study concerning customer distributions, the problem involves conceptions, trade routes, thresholds, and a multitude of other interrelated questions. The data must always be analyzed with these things in mind. But first, the data must be obtained as effectively as possible.

Hypothesis

It is the hypothesis of this research study that it is possible to sample customer origin without the use of questionnaires or interviews in order to determine the distribution of customer origin as related to specific shopping centers. Furthermore, the data obtained from such a method has the potential to be utilized to make inferences concerning variables that could effect the distribution of customers to various shopping centers.

Method

The technique of observation has been defined as the actual physical process by which the information is gathered. The choices of these techniques are classified as 1) self-surveys, 2) interviews, and 3) direct inspection (6). The system used in this study is direct field inspection. Bias and Cost are the two criteria that must be considered in the choice of techniques of observation. These two criteria are often interrelated and a balance should be made between the two.

On this basis, Indiana is an opportune state for direct field inspection required in a study such as this one. Each community within the state is assigned a prefix of specific automobile license plate numbers that are unique in their community. Therefore, the origin of motor vehicles on the parking lot of a shopping center is easily determined from the license plate number. This method of data collection immediately eliminates one of the two criteria for choice of

sections are charted and labeled. Each section consists of an equal number of rows of cars. Cars were selected from rows in each section. Every third and fourth car is selected in every row so that each section ended up with an equal amount of license numbers.

The purpose for gathering information by sections is to enable the license numbers to be easily recorded at different times during the day without an overlap. Odd numbered sections are investigated at the same hour, whereas, even numbered sections, in the same shopping center, are investigated three hours later. For example, sections one, three, and five in Honeycreek Mall are sampled at one o'clock on a given day. Sections one, three, and five are sampled at North Plaza one hour later at two o'clock. Sections one, three, and five are finally sampled at K-Mart Plaza at three o'clock, and K-Mart Plaza at six o'clock. When the day is over, a total of one hundred license numbers per shopping center are recorded, covering two different times during the day for each center.

The starting order of the sequence of shopping centers is also rotated by week. The one o'clock samplings start at Honeycreek Mall the first week, North Plaza the second week, and K-Mart Plaza the last week. This rotation makes sure that one shopping center is not always sampled at the same two times during the day. The exact parking spaces sampled within each row of a section are also rotated to avoid possible overlap.

All of this careful rotation is planned for the purpose of eliminating as much bias as possible in the sampling. Different times of the day are incorporated in the sampling to assure a more even sample of all customers. The parking lots are charted out and sampled to insure an even sample from all parts of the lot. Different hours are rotated to insure a sample of customers from as many times as possible. After three weeks of this rigorous sampling design, a sample of 1819 license numbers from the three shopping centers were obtained.

The results of this procedure are displayed only to emphasize the output of the method. The arrangement of the material is displayed only to relate possible analysis that could be made. Since analysis was not the main objective in this research, the results are merely displayed and not rigorously tested or subjected to all variables.

The data obtained from this sample are divided up into the classes: Honeycreek Mall, North Plaza, and K-Mart Plaza. Several categories are found for each of the shopping centers. License tags are then grouped into the categories. These categories are based on conglomerations of license plate areas and for Honeycreek Mall consist of: 1) Illinois license numbers, 2) Terre Haute license numbers, 3) License numbers from South of Terre Haute, and 4) other assorted license numbers from all over the state of Indiana.

The license numbers from North Plaza are divided into 1) those customers from Terre Haute, 2) those customers from north of Terre Haute, and 3) customers from other parts of the state. K-Mart Plaza license numbers are divided into 1) Terre Haute customers 2) those numbers from east of Terre Haute, and 3) other assorted numbers from various parts of the state. It must be noticed that a category of numbers is included for the direction that each

shopping center is from Terre Haute (north for North Plaza, South for Honeycreek, and east for K-Mart Plaza).

The results of the study are then tabulated according to the categories for each shopping center. Honeycreek Mall has a total of 614 license numbers collected over the three week period. Out of these 614 numbers, 17% or 101 came from communities south of Terre Haute. Another 156 or 25% of the total came from Illinois to Honeycreek Mall. A total of 208 of the 614 came from Terre Haute which made up 34%. The other 149 license tags constituted 24% of the total 614 and came from the final category assigned to "other" communities in the state.

A total of 607 license numbers were collected over the three week period for North Plaza. The first category that these are divided into is the customers from north of Terre Haute. This category makes up 25%, or 151 customers out of 607 samples. Another 65% or 393 license numbers came from Terre Haute to shop at North Plaza. The final category is other cars coming from the other areas of the state, and makes up 10% or 63 of the total 607 license numbers.

The final shopping center, K-Mart Plaza, has a total of 598 license numbers collected over the three week period. Of these, 109, or 18%, are from communities to the east of Terre Haute. A total of 338, or 65% are from Terre Haute. The final category of K-Mart Plaza is those license numbers from other parts of the state not included in the other categories which consisted of 17% or 101 of the total 598. It must be remembered at this point that all of the above percentages are based upon the total number of license numbers per shopping center, and not the total of 1819 samples for the entire study.

The data so far can be summarized in the following chart (Table I). This material was displayed as totals for all three weeks.

TABLE I License Number Distributions
Total = 1819

	No.	%
Total from South coming to Honeycreek	101	17
Total Illinois coming to Honeycreek	156	25
Total Terre Haute coming to Honeycreek	208	34
Total other coming to Honeycreek	149	24
Total	614	100%
Total from North coming to North Plaza	151	25
Total from Terre Haute coming to North Plaza	393	65
Total other coming to North Plaza	63	10
Total	607	100%
Total from East coming to K-Mart Plaza	109	18
Total from Terre Haute coming to K-Mart	388	65
Total other coming to K-Mart Plaza	101	17
Total	598	100%

The basic trends can be extracted from this display, but other presentations also tend to bring out further information that could be analyzed.

The following chart (Table II) breaks the data down into week one, two, and three for each shopping center. The numbers are then broken down to the specific day (Saturday or Sunday) in each weekend.

TABLE II *Display of Raw Data by Weeks*

	Week #1			Week #2			Week #3		
	Sat.	Sun.	total	Sat.	Sun.	total	Sat.	Sun.	total
Total from South coming to Honeycreek	16	16	32	13	20	33	16	20	36
Total from Illinois coming to Honeycreek	16	14	30	32	26	58	36	32	68
Total from Terre Haute coming to Honeycreek	39	46	85	30	35	65	29	29	58
Total other coming to Honeycreek	25	28	53	26	29	55	21	20	41
Total from North coming to North Plaza	21	21	42	36	25	61	27	21	48
Total from Terre Haute coming to North Plaza	66	73	139	58	73	121	62	71	133
Total other coming to North Plaza	14	9	23	6	12	18	9	13	22
Total from East coming to K-Mart Plaza	16	18	34	12	21	33	17	25	42
Total from Terre Haute coming to K-Mart Plaza	70	66	136	73	58	131	63	58	121
Total other coming to K-Mart Plaza	15	15	30	16	20	36	22	13	35

The Analysis

In order to properly analyze the data, several economic concepts must be kept in mind. All of these concepts, and the hypothesis of this paper, are based on the assumption that individual consumers behave, on the average, in a predictable fashion. The consumer travel behavior of individuals is related to 1) the characteristics of individuals, 2) the way in which they perceive the array of places to which they may travel, and 3) the individuals' image of the total friction of distance between places of residence and the places where the individual may acquire the desired good or service (3).

The first fact to be analyzed was the relationship of the customers which came from the areas closest a particular shopping center. Customers usually appear to make systematic choices of the closest centers offering the goods they need (2). We would then assume that the communities from the south would patronize Honeycreek Mall; communities from the north patronize North Plaza, and communities from the south patronize Honeycreek mall, and communities from the east patronize K-Mart Plaza. When examined from the sampling data, we find that of a total of 614 license numbers taken at Honeycreek Mall, 406 are non-Terre Haute customers. Out of the 614 customers, only 17% or 101 came from the south to shop at Honeycreek Mall. The rest of the non-Terre Haute customers came from Illinois 25% or 156 and other assorted areas in Indiana 24% or 149. The high number of Illinois customers is probably due to the close proximity of the state of Illinois to Honeycreek Mall and also to the close proximity of Honeycreek Mall to a major highway system (Interstate 70) that runs through Illinois into Indiana.

North Plaza seems to follow the theory of closer distance more closely than Honeycreek Mall. The non-Terre Haute customers at North Plaza amount to 214 out of 617 total over the three week period. Of the 607 total, 25% or 151 came from the north of Terre Haute. Only 10% of the 607 total came from areas other

than Terre Haute and north of Terre Haute. In other words, 70% of the non-Terre Haute customers are from the north of Terre Haute.

K-Mart Plaza had a total of 210 or 35% out of 598 that are non-Terre Haute customers. Of the 598 total customers, 18% came from the east to visit K-Mart and 17% came from places other than Terre Haute or east of Terre Haute to shop at K-Mart. In other words, 52% of the non-Terre Haute customers are from the east and 48% from other areas in Indiana.

It, therefore, is evident that the bulk of the non-Terre Haute customers are not necessarily from the nearby southern communities for Honeycreek and the nearby eastern communities for K-Mart Plaza. It is true, however, that a larger percentage of the non-Terre Haute customers shopping at North Plaza are from the nearby northern communities. The observation that Honeycreek Plaza and K-Mark Plaza tend not to attract a larger customer trade from the closer southern and eastern (respectively) communities is not distinct only to this case. Other studies indicate that individuals do not necessarily shop at the nearest center where a good is available (3).

Several reasons can be presented for the distribution of customers at Honeycreek and K-Mart Plaza. Shopping centers can carry a definite delimitation and classification (7). Shopping centers vary in trading characteristics and organization so that people perceive centers as more attractive because of more or different choices and because of associated activities. Honeycreek Mall attracts a more even percentage from the four categories than the other shopping centers. (17%, 25%, 34%, 24% in the four categories) This could be due to a greater diversification of goods as well as a location in an easily accessible area. K-Mart Plaza offers a different phenomena that has been known to change the shortest distance tendencies (1). The presence of a discount center in the Plaza tends to equal out the percentages of people coming from all areas, as can be seen in the data (East 18%, other 17%). The different department stores "magnets" of these shopping centers also tends to spread out the distributions because people patronize department stores according to their own personal preferences (1).

Another interesting analysis from this data can be drawn from the Terre Haute customers. Since they are relatively near all three centers, it is interesting to note how they tend to patronize these three shopping centers. Honeycreek has the least Terre Haute customers of the three shopping centers (34% of the total license numbers), North Plaza and K-Mart Plaza have equal Terre Haute patronages (65% for each). This could be true because Terre Haute people tend to avoid the busier areas (Honeycreek) when they seek goods that will be found elsewhere with less confusion. Also the K-Mart discount store offers a quick, low cost facility with east access for Terre Haute customers.

Conclusions

In conclusion, it can be said that the hypothesis of this paper has been proven as valid. Using field techniques, it is possible to determine customer origin characteristics for the specifically defined shopping centers and possible analysis methods can be utilized with the data obtained. The purpose of the

study is to present the method and reasons for using the method, as well as to present an example of the process actually being utilized.

The utility of this study can be applied in several ways. Shopping center stores would be interested in advertising in the areas that tend to have more customers, or they might be interested in concentrating on areas that do not support them as much as might be expected. Any store that is interested in making a profit will be interested in where its customers come from. Honeycreek Mall had a customer range that is more equally divided among the different areas, possibly indicating a regional shopping center, so store owners should know how to avoid limiting their advertising to one specific area. North Plaza attracts mostly Terre Haute customers, but emphasis should also be put on areas north of Terre Haute. K-Mart Plaza, for the most part, has most of its customers from Terre Haute, so the emphasis should be placed within Terre Haute. Other utilities of this study can be found in transportation planning, new store development, and new housing development.

Any good research problem that is investigated properly, always gives rise to other problems that are not in the scope of the research, but can be investigated in another study. Several problems can be drawn from this study. Is there a definite economic class system that is exhibited between the different centers? Do customers from different areas have specific times that they tend to patronize the shopping centers within? Is it feasible to establish a shopping center in west Terre Haute? These questions and several more require specific studies and further applications of field techniques.

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MICROBIOLOGY & MOLECULAR BIOLOGY

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Abstracts

On the Serology of *Bacteroides* species. WILLIAM W. BALDWIN, ALEXANDER A. STEMER and MARSHALL E. LANDAY, Indiana University School of Medicine, Northwest Center for Medical Education, Gary, Indiana 46408 and St. Margaret Hospital, Hammond, Indiana 46320.——Antiserum prepared in New Zealand white rabbits by giving six intravenous injections of formalin fixed whole cells of *Bacteroides fragilis* showed numerous bands when reacted with *B. fragilis* antigen in Ouchterlony plates. This antigen was prepared by breaking *B. fragilis* with glass beads and using the soluble supernatant after centrifugation. The above antiserum also reacted with *Bacteroides thetaiotaomicron* antigen, prepared as above, and showed one band of apparent identity with *B. fragilis*. The band of apparent identity for both organisms was lost if the antigens were heated at 80°C for 30 minutes before reacting with antibody. Antiserum prepared as above against *B. thetaiotaomicron* did not react with *B. fragilis* antigen. A precipitin curve was performed using anti- *B. fragilis* antiserum and *B. thetaiotaomicron* antigen and optimal precipitate was obtained using an antibody to antigen ratio of 3:1. This ratio was used to precipitate a quantity of *B. thetaiotaomicron* heat labile antigen. This precipitate was dissolved in 1.5M acetic acid and was separated by gel filtration on an EM gel 1000 A column. Two peaks, measured at 280 nm, were seen on elution of the column with 1.5M acetic acid. One peak found at the void volume gave a reaction, after dialysis and concentration, in Ouchterlony plates like the heat labile antigen above. The other peak did not react with *B. fragilis* antiserum.

Investigation of Bacterial Mouth Flora of Ball State University Students. M. LANGONA, D. HENDRICKSON, J. HENDRIX, D. GOBBLE, Ball State University, Muncie, Indiana 47306.——The occurrence of pathogenic and non-pathogenic bacteria isolated from the throats of thirty Ball State University students was investigated in a surveillance study utilizing weekly throat swabbings. The study was conducted during the Winter Quarter of the 1977-78 academic year. The reference population was divided into three groups; the control; the semi-control; and the experimental group. The experimental group was exposed to numerous bacterial pathogens and non-pathogens bi-weekly during one academic quarter. The objective of the study was to determine if the throat flora of the individuals within the experimental group underwent changes in relation to the bacterial organisms to which the individual was exposed. The results of

the study indicated that bacterial changes did not occur within the throat region following bacterial exposure.

Implications of Undesired Effects of Antibiotic Use in Rodents. D. MADSEN, B. WOSTMANN, and M. BEAVER, Lobund Laboratory, University of Notre Dame, Notre Dame, Indiana 46556.——In murine rodents, such as the laboratory rat, the major fecal, secondary bile acids (BAs) are hyodeoxy-cholic (HDC) and ω -muricholic (ω -MC), both being derived from the BA, β -muricholic acid. The ratio of HDC/ ω -MC in feces is a sensitive indicator of perturbations of the intestinal microecology. For example, treatment of rats with aureomycin, penicillin, neomycin or polymyxin for only 5 days results in a lowering of the HDC/ ω -MC ratio from the control value of around 1.9 to values below 0.90. This effect is termed the "HDC/ ω -MC shift", and results in ω -MC replacing HDC as the major fecal BA. Two undesired "side effects" of antibiotic treatment have been observed. The first is a persistence of the lowered HDC/ ω -MC ratio for at least 4 months after cessation of treatment. The second side effect, seen to date only with polymyxin, is a gradual spreading of the HDC/ ω -MC shift to untreated control rats housed nearby; this is seen only after 1-2 months beyond treatment of the experimental animals. Implications of the results are that antibiotically altered microflora can persist long after their induction, and undesired floras may be produced by such treatment. Spreading of the phenomenon suggests that the altered flora can spread to control animals and express their new potential, thus changing the microecology of untreated animals.

Isolation of a Calcium Binding Protein from Cat Brain and Spinal Cord and its Comparison with the Fast Transported Calcium Binding Protein of Cat Sciatic Nerve. ZAFAR IQBAL, Department of Physiology and Medical Biophysics Program, Indiana University School of Medicine, Indianapolis, Indiana 46202.——Fast axoplasmic transport of calcium in cat sciatic nerve has been shown to be associated with a calcium binding protein (CaBP) of 15,000 daltons (Iqbal & Ochs, J. Neurochem. 31:409-418, 1978). Using a column gel chromatographic techniques, brain and spinal cord of cat have been found to contain a protein of molecular weight similar to that of nerve CaBP. The tissue (brain or spinal cord) was homogenized in a 10 mM phosphate buffer of pH 7.5, the homogenate centrifuged at 105,000 g for 1 hour and the supernatant so obtained was concentrated by freeze drying. The proteins in the supernatant fractions were fractionized on a column (2.4 x 40 cm) packed with Sephadex G-100 using an automatic fraction collector. The column was equilibrated and eluted with a 10 mM phosphate buffer of pH 7.5 and the first peak, Peak I, was further analyzed on BioGel A5m (1.6 x 60 cm) using the same system as with Sephadex G-100 column. The fractions corresponding to the third peak, I_c, were pooled, reduced in volume by lyophilization and dialyzed against 0.1% glycine. The properties of the proteins isolated from brain and spinal cord were compared with that of nerve CaBP by electrophoresis on 10% acrylamide gels with or without 0.1% SDS. The proteins of brain and spinal cord were found to comigrate with the nerve CaBP suggesting that all three proteins are similar in nature as regards to their molecular weight and electrophoretic mobilities. Supported by the NIH grant PHS RO1 NS 8706-09.

An Unusual Organism Suggestive of *Beijerinckia* spp. in Stream Leaf-Litter.

GARY W. PETTIBONE, Department of Biology, Bowling Green State University, Bowling Green, Ohio 43403 and DONALD A. HENDRICKSON, Department of Biology, Ball State University, Muncie, Indiana 47306.——An organism suggestive of the genus *Beijerinckia* was found in association with stream leaf-litter in Bell Creek (Delaware County, Indiana). Leaf material was recovered from the stream and homogenized aseptically in the laboratory. Serial dilutions were performed and triplicate spread plates were placed to extinction on Tryptone Glucose Yeast Extract Agar. Incubation was at ambient temperatures (20-24° C.) for up to twenty-one days. Typically convoluted colonies possessing a hard tenacious slime were observed on all plates exhibiting bacterial growth. These colonies comprised between twenty and thirty per cent of the heterotrophic bacterial population recovered by the spread plate technique. The cells were purified and tentatively identified as members of the genus *Beijerinckia*. This organism is a non-symbiotic nitrogen fixer and is usually considered to be restricted to acidic tropical soils although it has been isolated from temperate regions in a few cases. The presence of this organism in stream leaf-litter could be important in supplying fixed nitrogen for cellulose decomposing microorganisms.

Heterotrophic Bacterial Populations Associated With Leaf-Litter in an Indiana Stream

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Introduction

Temperate region woodland streams have been shown to be heterotrophic in nature and rely primarily upon allochthonous matter as a primary source of energy (8) (9) (17) (19) (20) (24). A large portion of this allochthonous material arrives in the form of autumn-shed leaves from the surrounding watershed (9) (18) (25). In addition, estimates of leaf-litter production in different woodlands ranges from 2.5 to 6.8 metric tons/ha/yr (3), suggesting that the leaf-litter entering streams is substantial, especially in the autumn of the year.

The fate of leaves after they have entered a stream has received some attention of late. Initially, they float on the water surface and move in response to wind and water currents. Gradually, they become waterlogged and sink to the bottom accumulating in shallow margins and the downstream edges of pools. They also tend to aggregate or clump against obstacles that are located in the stream and in this respect a riffle is an excellent obstruction with which to trap leaves.

It has been documented that leaves are leached of many of their soluble products after they enter the stream, usually within the first twenty-four hours (14) (16). After leaching, a period of nitrogen enrichment occurs which has been attributed to the microbial colonization of the leaf-litter (13) (14) (16) (18). The colonization of leaf-litter by bacteria and fungi has been called "conditioning" and is important in preparing the leaf material as a palatable food source for macroinvertebrates (2) (5) (21).

The role of many macroinvertebrates in leaf-litter degradation has been identified and categorized (5). However, little attention has been paid to the bacterial species that are present in the leaf masses. The large influx of leaves in the fall of the year can lead to large accumulations of leaf-litter all of which are subject to bacterial colonization. These aggregations are in effect unique entities in terms of resources and environments and their size influences the conditions which exist within each leaf mass (2). Therefore, physical factors such as dissolved oxygen and water movements are effected. The latter parameter may be important in terms of the physical disruption of the leaf material since sediments in transport may act as abrasives. The former condition will greatly influence the microbial population and activity and is therefore of major interest in terms of the bacterial influence on leaf-litter decomposition.

Considering the fact that continual packing of leaf material occurs during

the autumn it seems reasonable to assume that reduced oxygen conditions, and possibly anaerobic conditions, are generated through the continued accumulation of new material. These conditions would, by their nature, limit the types of organisms present to those that could function and survive in microaerophilic or anaerobic environments. This can be observed in terrestrial biomes in waterlogged soils where aerobic fungi and bacteria are unable to develop due to prevailing anaerobic conditions (27). Under these circumstances, the anaerobic bacteria capable of degrading non-soluble products such as cellulose and lignin would seem to be an important factor in leaf-litter decomposition. It has been suggested that the spore-forming *Clostridium* are the dominant cellulolytic bacteria in anaerobic aquatic environments (12).

Thus, the autumn leaf packs appear to sustain a dynamic mosaic community of various microbial and invertebrate species colonizing the leaf-litter. This study was undertaken to: 1) examine the dominant heterotrophic bacterial species associated with the leaf-litter and 2) to determine if anaerobic cellulolytic bacteria were present and possibly functioning in leaf-litter decomposition.

Materials and Methods

Leaves of the sugar maple (*Acer saccharum*) were collected and placed in 8 x 12 inch nylon mesh bags (mesh size: 2300 μ bs 312 μ). In September, 1976 the bags were placed above a riffle in Bell Creek, Delaware County, Indiana. Duplicate bags were attached to stakes by nylon fishing line and allowed to float downstream to become a part of the developing leaf mass. The leaf bags were sampled approximately monthly from December, 1976 to March, 1977 and analyzed for aerobic heterotrophic bacteria, total anaerobic heterotrophic bacteria, and anaerobic bacteria capable of cellulose decomposition. The analysis was performed at Ball State University, Muncie, Indiana.

When the leaf bags were sampled they were brought back to the laboratory aseptically and under both aerobic and reduced oxygen conditions. In the laboratory, samples were prepared by homogenizing the leaf material in sterile phosphate buffer (pH 7.2) at a 1:10 dilution (V/V). Homogenization was performed in a Waring Blender set at high speed for two minutes. Leaves were homogenized aerobically and anaerobically and cultured under both oxygen conditions.

Aerobic isolation consisted of spread plating decimal dilutions of prepared sample to extinction on triplicate plates of Tryptone Glucose Yeast Extract Agar (TGYEA) and incubation at ambient temperatures (20-24°C.). Typical bacterial colonies were purified through further transfers on TGYEA and subjected to biochemical examination. All incubation temperatures were at ambient temperature except those tests which required special incubation procedures.

The anaerobic isolations procedures were performed as above but were carried out in a bacteriological transfer hood (Lab. Con. Co.) which had been altered to maintain an anaerobic atmosphere. The hood was evacuated and flooded with CO₂ several times before any operations were performed. A

constant stream of CO₂ was allowed to run during the isolation procedures to insure a positive pressure inside the chamber. The TGYEA plates were incubated at ambient temperature in gas-pack jars (B.B.L.). Typical colonies were purified and transferred to two TGYEA plates. One plate was incubated aerobically and the other was incubated anaerobically. Colonies growing on aerobic plates were picked and transferred to differential media for biochemical identification. Colonies growing only on anaerobic plates were gram stained and inoculated into chopped meat-glucose medium and peptone-yeast extract medium for identification by gas-liquid-chromatography and by the V.P.I. system for culturing and identifying anaerobes.

Anaerobic cellulolytic bacteria were investigated using a multiple tube test with Imshenetskii medium No. 1. This medium was prepared according to Rodina (23) with the notable exception that the cellulose was homogenized into a slurry (1.5% cellulose). Whatman #1 filter paper was used as a source of cellulose. Each tube in the series contained approximately ten milliliters of cellulose medium. All tubes were overlaid with sterile heavy weight mineral oil after inoculation to insure that anaerobic conditions were maintained.

After approximately two weeks inocula from each tube in the series were transferred to another set of tubes containing the same medium and again overlaid with sterile oil. The tubes that eventually showed a marked reduction in the amount of cellulose present were considered positive. Enumeration was carried out according to the 14th edition of *Standard Methods for the Examination of Water Wastewater* for the multiple tube tests (1). Inocula from each reduced tube were streaked onto TYGEA plates and incubated anaerobically. Colonies were purified and transferred to two TGYEA plates and incubated as above.

Aerobic isolation on non-selective medium

Typical bacterial colonies from the higher dilution TGYEA plates were identified biochemically. Pigmented bacteria of the genus *Flavobacterium* were the most numerous on nearly all plates and comprised between thirty-five and forty-five percent of the recovered bacterial population. Members of the genus *Pseudomonas* were also dominant and comprised between thirty and forty percent of the recovered population. Another organism suggestive of the genus *Beijerinckia* was isolated routinely on all high dilution plates and comprised between twenty and thirty percent of the bacterial population recovered by the spread plate technique.

Members of the genera *Bacillus*, *Achromobacter*, *Chromobacterium*, and various members of the family Enterobacteriaceae, especially *Klebsiella*, were isolated routinely at most lower dilutions. Enumeration of aerobic bacteria demonstrated an average maximum value of 1.5×10^8 bacteria/ml in the March sample. Low values of 9.0×10^5 bacteria/ml were recorded in January.

Anaerobic isolation on non-selective medium

A number of facultative bacteria were isolated on TGYEA under anaerobic conditions. Almost all of the organisms represented in this group were members of the genus *Pseudomonas*. Two of these were tentatively identified as *Ps. effusa* and *Ps. mira*, both of which are reported to attach cellulose according to Breed

et al. (4). Members of the genus *Klebsiella* were also isolated under these conditions and were the only other facultative organisms recovered using this technique. Plate counts from this procedure ranged from 6.0 to 10^4 bacteria/ml in January to 1.8×10^5 bacteria/ml in March.

Anaerobic bacteria were also isolated which were capable of growth on TGYEA after the initial inoculation but failed to grow upon subsequent transfers. Gram stains of these organisms revealed that they were gram positive rods or filaments. Gram stains of other anaerobes that grew weakly or well upon transfer showed that most were gram positive rods with terminal spores which is suggestive of the genus *Clostridium*. Purified isolates of these organisms were subjected to gas-liquid-chromatography. All such cultures showed a very large butyric acid peak and a somewhat smaller acetic acid peak. These data are again suggestive of the genus *Clostridium*. Biochemical tests performed on these organisms with the V.P.I. anaerobic system did not prove useful in identifying the bacteria to species.

Anaerobic isolation in cellulose medium

Several facultative organisms were isolated in the cellulose medium including *Ps. effusa* and *Ps. mira* along with various members of the family Enterobacteriaceae. However, all tubes which demonstrated a reduced cellulose content were found to contain gram positive sporogenous rods. The same characteristic acid peaks were obtained from analysis of these organisms as described above for the sporeforming bacteria isolated anaerobically on TGYEA. The number of cellulolytic anaerobes/ml ranged from a low of 93 in December and March to a high of 150 in January.

A list of the dominant and common genera of bacteria that were routinely isolated during the investigation is shown in Table I.

Discussion

The identification and enumeration procedures used in this investigation revealed a variety of bacteria associated with the leaf-litter habitat. Aerobic bacterial plate counts reached 1.5×10^8 bacteria/ml which compares favorably with the counts obtained by other investigators (15) (22). Obviously the method employed enumerates only those organisms that are nutritionally and competitively able to develop on the TGYEA plates. However, the developing microorganisms, as well as those that are not culturable, utilize common substrates in the stream and should respond similarly to environmental changes. (Witkamp) (28) has reported on the validity of the serial dilution technique in relation to counts from terrestrial leaf-litter.

It should be realized that the enumeration of microorganisms cannot act as a measure of their importance in the process of decomposition. It has been suggested that there may be no relationship between the number of microorganisms present and metabolic rate (10) and that the importance of bacteria in organic decomposition is determined by the latter parameter rather than the former (28). This investigation does not attempt to equate the mere presence of bacteria in the leaf-litter with a direct function in regards to decomposition. Nevertheless, the complexity of the microbial colonization of

large leaf masses suggests a "community" concept and offers the potential for both direct and indirect interactions among the community members.

TABLE I *Dominant and common genera of bacteria isolated from leaf-litter in Bell Creek, Delaware County, Indiana, from December, 1976 to March, 1977.*

Dominant Genera	Common Genera
Flavobacterium	Enterobacter
Pseudomonas	Citrobacter
Beijerinckia-like organism of unknown taxonomy	Klebsiella
	Bacillus
	Achromobacter
	Chromobacterium
	Clostridium

Most of the bacteria isolated during this investigation were not capable of degrading the cellulose of lignins found in large quantities in leaves. The dominant genera isolated from the leaf-litter were *Flavobacterium*, *Pseudomonas*, and a *Beijerinckia*-like organism of unknown taxonomy. These data are similar to those of Suberkropp and Klug (26) who found *Flavobacterium* and *Pseudomonas* to be among the major genera associated with oak and hickory leaves in a woodland stream. *Flavobacterium* were also found to be a dominant member of the microbial population associated with organic matter in transport in streams (6) and an organism identified as *Flavobacterium* has been isolated which clearly stimulated the growth of the cellulolytic bacterium *Sporocytophaga* in mixed culture (11). This type of association may be a factor in the roles played by bacteria in leaf-litter decomposition.

Similar indirect associations may be hypothesized for other non-cellulolytic bacteria found to be present in the leaf-litter. the *Beijerinckia*-like organism is suspected of fixing atmospheric nitrogen and might possibly provide fixed nitrogen to the community. This function might also be attributed to the *Enterobacter* and *Klebsiella*, which were found routinely, since members of these genera have been implicated in nitrogen fixation (7). The various members of the genus *Pseudomonas* may also impact some indirect influence on the amount of leaf material which is degraded. Certain members of the genus *Bacillus* have also been found to degrade cellulose and the isolation of this genus from the leaf-litter should not be considered unimportant.

The scope of this study and the complexity of the microbial community within the leaf-litter precludes any detailed analysis of specific relationships. However, we do emphasize that the potential for commensal, mutual, and synergistic relationships exists.

When the leaf-litter habitat is viewed with respect to community interactions it is possible that a small number of bacteria could influence decomposition more readily than a larger number of less active species. A case in point is the relatively small number of cellulolytic bacteria found in the leaf mass. No attempt was made to directly measure the importance of these bacteria

in the decomposition process. However, the continued accumulation and packing of leaf-litter should result in microenvironments capable of sustaining anaerobic or facultative organisms. Therefore, it is assumed that if the organisms were routinely isolated from the leaf mass then the proper conditions exist in the field to allow them to be metabolically active. Since gram positive sporogenous rods (*Clostridium* spp.) were found in all tubes showing cellulose reduction, it is probable that these organisms are also active under field conditions.

These data by no means represent the total bacterial composition of the leaf-litter habitat. Only the dominant species associated with this particular leaf mass have been identified. Since only those bacteria that grew primarily on TGYEA were isolated many bacterial species were not cultivated. Cellulolytic members of the genera *Cytophaga* and *Sporocytophaga* have been isolated from leaf-litter in other investigations (22) (26) but were probably not selected for by the methods employed here. It must be emphasized that we are not suggesting that the bacteria are the most important organisms involved in leaf-litter decomposition. Other data suggest that the fungi might be more important, at least initially (16) (26). However, the value of bacteria associated with stream leaf-litter decomposition should not be overlooked, especially in the microenvironments that are more suited for their colonization. Hopefully, further studies will be performed relative to the function and relationships of bacteria in the overall energy budget of heterotrophic streams.

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PHYSICS

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Abstracts

In Situ Measurements of Cutaneous Fluorescence. TORSTEN ALVAGER, WALTER X. BALCAVAGE and WEICHEN TU*, Department of Physics and Terre Haute Center for Medical Education, Indiana State University, Terre Haute, Indiana 47809.——In situ fluorescence from skin of rats and humans has been studied using a newly developed light guide method. The technique is of importance as a possible diagnostic tool, since pathological states in biomaterial are often accompanied by the formation of tissue areas with altered fluorescence properties not necessarily caused by local phenomena. To obtain a first view of the possible use of this biophysical method, the study has been restricted to observations of only two fluorophores: proteins and so-called age pigments at excitation wavelengths 295 nm and 398 nm respectively. Results from measurements on humans as well as a series of observations on rats as a function of age will be discussed.

Production of artificial spin centers on chars. S. MROZOWSKI, Department of Physics and Astronomy, Ball State University, Muncie, Indiana 47306.——It was previously shown by several people that artificial spin centers (A-centers) can be made on chars and carbon blacks, by heating these materials to 700-900° in atmospheres of various gases under high pressure. The author has found that in heating a polyvinylidene chloride (PVDC) char reacts with its own evolved gas, even at quite low pressure, producing A-centers. In this work gas evolved from PVDC (mostly a mixture of HCl and Cl₂) was used to make A-centers on variously heattreated sugar chars and P33 carbon blacks. The production of A-centers and their destruction by thermal anneal in vacuo were studied; it was found that such A-centers can be made only as long as a carbon contains on peripheries some other organic components (H, N, O, etc.). Temperature dependence of the ESR absorption, its separation into air sensitive and air insensitive components and other effects will be discussed.

IUE Satellite Observations of Several Optically Violent Extragalactic Radio Sources. ROGER L. SCOTT, Department of Physics and Astronomy, Ball State University, Muncie, Indiana 47306, KAREN R. HACKNEY and RICHARD L. HACKNEY, Department of Physics and Astronomy, Western Kentucky University, Bowling Green, Kentucky 42101.——The IUE satellite is being used to study several BL Lacertae type objects in the ultraviolet below atmospheric cutoff. BL Lacertae objects exhibit the most violent optical and radio variability of the quasar class of objects. These rapid variables may represent a transitional phase between so called "peculiar galaxies" and the

quasar phenomenon. A brief discussion of preliminary results and instrumentation will be given. The observations were made at Goddard Space Flight Center and were supported by NASA Grant NSG 5238.

A Microcomputer Based Electronic Kinetic Sculpture. B. G. FORDHAM and G. P. THOMAS, Department of Physics and Astronomy, Ball State University, Muncie, Indiana 47306.——An SDK-85 microcomputer was programmed to control an incandescent light and analog sound source system, that form the nucleus of an optical-acoustical kinetic sculpture piece. Physical design, construction, operation, and control of this sculpture will be discussed with emphasis on such topics as the need for the advantage of computer control and interfacing details.

Using a Kim-1 Microprocessor as a Data Logger for Temperature Measurements in the Real World. VINCENT A. DINOTO, JR.——A brief discussion of a 6502 based microprocessor system for monitoring temperatures in the real world and store data over a short period of time. The 6502 based Kim-1 is interfaced to 3911 temperature probes which are frequency dependent with temperature. The software controlling programs can be designed to monitor as many as 15 probes or as few as one, with our applications we will not be using more than 8. The probes are calibrated independent of each other and each probe has its own linear calibration curve over our temperature ranges. Each calibration curve is repeatable for that probe. The probes have many real world applications with river temperature monitoring and energy losses being just two that are currently being tested.

Thermal Analysis of a Wind-Supplemented Residential Hot Water System. MICHAEL D. LOYD and RONALD M. COSBY, Department of Physics and Astronomy, Ball State University, Muncie, Indiana 47306.——A wind-supplemented residential hot water system was analyzed with general conditions established under which a wind-thermal transducer provides a useful heat source for this application. Example data was generated using a computer program modelling a vertical, composite cylindrical water tank simultaneously experiencing convective heat loss and wind-derived heat input under a variety of ambient conditions. Parameters varied included insulation thickness and quality, windmill size and conversion efficiency, and ambient windspeed and temperature. For appreciable heat gain in a well-insulated storage tank, an average windspeed greater than about 6 m/s was found necessary for a rotor with diameter 3.6 m and an overall efficiency of 0.25.

Design of an Optical Microprobe for In Situ Fluorescence¹

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Introduction

In situ biofluorescence measurements have in the past been restricted to surface fluorescence of tissue and organs. A rigid system of light beams have in general been employed to transport light between tissue and fluorometer. Recently, however, the technique has been developed into a more flexible method in which light guides have been substituted for the light beams (1, 4). This allows, for instance, for measurements of various changing metabolic states of cells in organs of an awake animal over a long period of time.

In this work we discuss the development of a light guide probe for measurement of fluorescence of cells not only on the surface of but also deep inside tissue and organs. It is clear that such a probe would allow studies not possible with a surface probe. For instance, the metabolic state of cells of different parts of a brain may and does vary considerably. A surface probe with a typical depth sensitivity of only a few hundred microns would not be capable of registering such a spatial variation. It is obvious that to avoid massive tissue damage in the use of such a probe it must necessarily be designed to have a needle-like structure with a small diameter. In this study probes having diameters in the range of 0.1 cm - 0.005 cm have been used and their light guide characteristics have been measured under various experimental conditions as described below.

Some Design Parameters

A schematic block diagram of an in situ fluorometer in use at Indiana State University (2) is shown in (Fig. 1). Excitation light from a conventional incandescent filament source or a laser is selected in a Schoeffel miniature monochromator and enters a Y-shaped light guide (a Schott uv transmission guide). The diameter of the shaft of this guide is 0.1 cm. As described below a glass (or quartz) rod shaped into a suitable microguide is adapted to the end part of the Y-shaped guide and transmits excitation light to the sample spot. Fluorescence light returns through the same microguide and part of this light is led through the second arm of the bifurcated guide to a second monochromator (Schoeffel double monochromator GM 200) through which fluorescence light is transmitted and recorded in a high sensitive photon counter (RCA 8850 photomultiplier).

¹Supported in part by a grant from ISU Research Committee

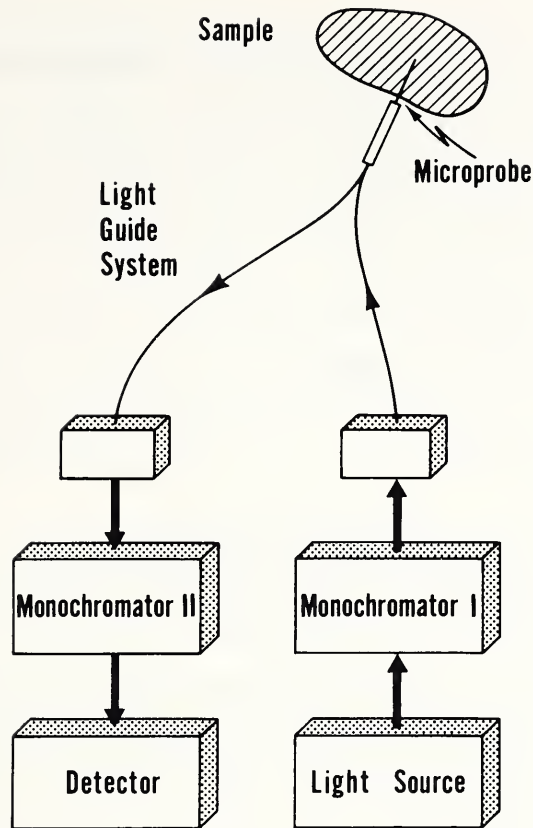


FIGURE 1. Block diagram of in situ fluorometer.

(Fig. 2) shows the principal design of the optical microprobe that has been found to be the most practical one in preliminary experiments. A pyrex glass (or quartz) rod is carefully heated and pulled by a micropipette puller to a straight tip forming the microprobe. This procedure allows for a tip size of less than $1\ \mu\text{m}$ in diameter which is a standard size used in electrophysiological experiments. To prevent light from entering or leaving the rod its surface is covered by a thin layer of reflecting paint, leaving the tip the only unexposed surface. The layer also serves as a reflecting medium giving the rod light guide properties. The microprobe is in direct contact with the main light guide. A certain amount of reflection does occur in the interface but in general this can be tolerated.

It should be noted that due to the good solidity of the glass rod no special mechanical support of the microprobe is needed. Also, the positioning of the probe in tissue can follow a procedure much like the procedure used in conventional electrophysiological investigations.

Since the microprobe is a tapered light guide the entrance angle of light will not be the same as the exit angle. This means that in general a certain amount of backreflection will occur with corresponding loss of transmission. To estimate the severity of backreflection consider (Fig. 3). A ray entering the front surface with angle α_1 is reflected inside the fiber and leaves the exit side surface at angle α_2 . If d_1 and d_2 are the diameters of the front and exit surfaces respectively and if only rays entering and leaving at the centers of the end surfaces are considered,

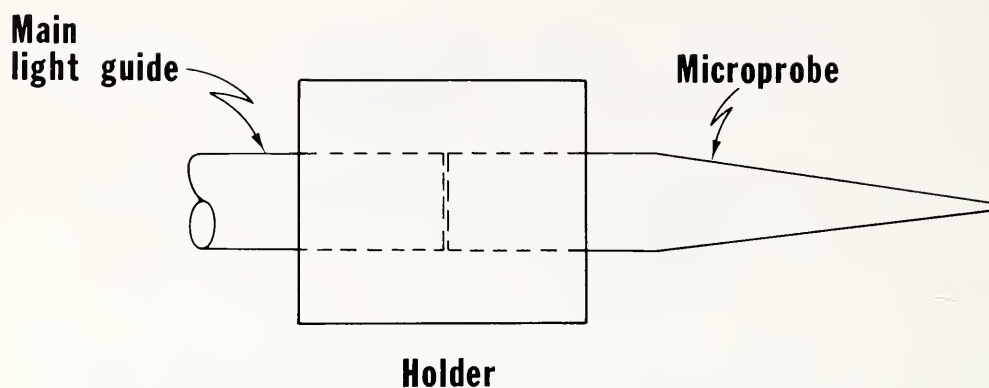


FIGURE 2. Optical microprobe.

elementary considerations show that the following relation holds for d_1 , d_2 , α_1 , and α_2

$$d_1 \sin \alpha_1 = d_2 \sin \alpha_2 \quad [1]$$

Choosing $\alpha_2 = 90^\circ$ we obtain the condition on α_1 for backreflection

$$\sin \alpha_1 = \frac{d_2}{d_1} \quad [2]$$

For instance, if $d_1 = 0.1$ cm and $d_2 = 0.01$ cm we find that $\alpha_1 = 5.7^\circ$. This means that for an entrance angle α_1 larger than 5.7° light transmitted along the fiber will be reflected back or leaving it before reaching the exit surface.

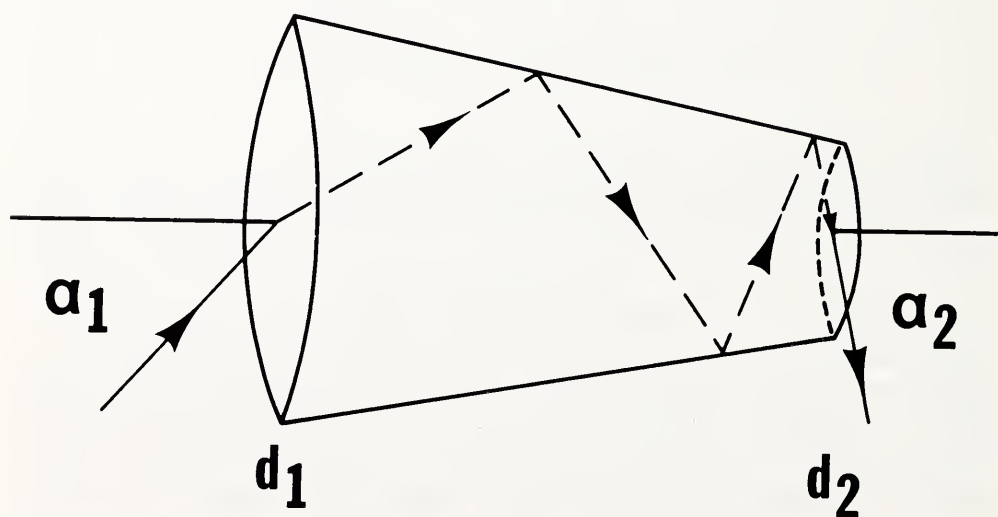


FIGURE 3. Ray diagram of tapered light guide.

If the light source of the microprobe system is of a conventional incandescent filament type, a spread of several degrees in beam divergence is usually present. A substantial backreflection and corresponding loss of transmission may therefore occur according to the discussion given above. On the other hand if the tapered light guide probe is fed light from a laser which has in general a very low spread in beam divergence ($\leq 0.1^\circ$) a low backreflection effect is expected. Choosing $\alpha_1 = 0.1^\circ$ and the d_1/d_2 ratio as stated previously ($d_1/d_2 = 10$) relation [1] indicates that the exit angle α_2 becomes approximately 1° , which still represents a small beam divergence. The angle amplification in the

tapered light guide will obviously not cause any significant problem until the diameter ratio d_1/d_2 approaches a factor of one hundred.

It should be noted that in the actual fluorometer (Fig. 1) the main light guide is bridging the path between the microprobe and the light source. However, the discussion given above about angle amplification in the tapered probe is still correct, since the main light guide is not tapered and conserves therefore the exit and entrance angles. Also, it should be noted that as far as angle amplification and transmission is concerned it is only the trip in the direction from main light guide to sample that is critical in the microprobe. Fluorescence light traveling in the opposite direction exhibits an angle demagnification and therefore does not give rise to any transmission losses through backreflections.

Results and Discussion

The two most important parameters to be determined experimentally for a microprobe are the cross-sectional profile of the light beam leaving the exit surface of the probe and the transmission of light in the probe.

The profile of the light beam has been measured in an experimental set-up consisting of a light detector and a small screen with a sharp edge which was movable, horizontally, across the opening slit of the detector. The microprobe was placed close to the detector slit with its center line perpendicular to the slit area and in such a position that the screen could block the light path to the detector. By recording the light intensity change in the detector as a function of the position of the screen, a measure of the integrated beam profile was obtained. (Fig. 4) gives two representative measurements of the horizontal beam profiles. The diameter of the entrance surface of the probes was in both cases equal to 0.1 cm while the diameters of the exit surface were measured in a microscope to be approximately 0.02 cm and 0.04 cm respectively. The light was supplied by a laser (TRW 83A Argon laser; beam divergence: 0.1°). The wavelength of light used was 480 nm. The distance between the probe and the detector slit was approximately 0.1 cm.

From a practical point of view the diameter of the cross-section of the beam as seen by the detector can be defined as the screen movement needed to change the detector intensity from 5% to 95% of maximum intensity. Using this definition, the curves in (Fig. 4) show that the beam diameter is about equal to the diameter of the exit surface of the microprobe. The light leaving the microprobe therefore forms a beam that is approximately parallel.

These measurements are in accordance with the theoretical results discussed in the previous section and show that by the use of a high quality light beam, the effect of angle amplification taking place in the tapered light guide can be neglected at least for tip diameters larger than 0.005 cm. As also indicated previously, this should not be true, however, if an incandescent filament source is used. Measurements with such a source performed according to the same scheme as those presented in (Fig. 4) show that a considerable beam divergence appears from the probe with corresponding transmission losses. The transmission of light for laser-fed microprobes is approximately 20% for an exit

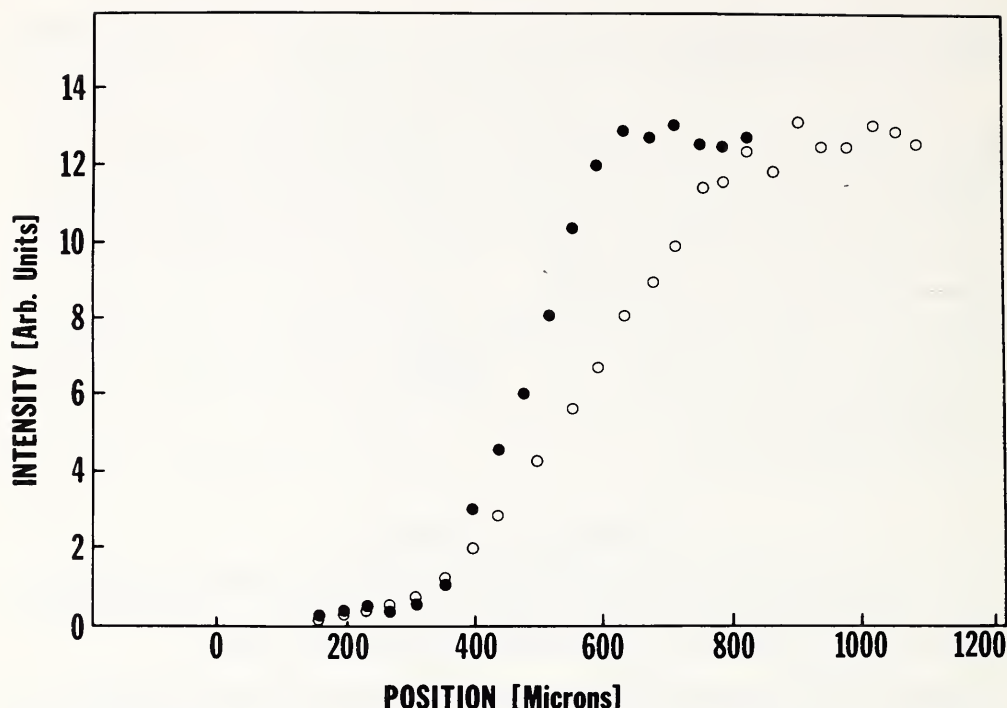


FIGURE 4. *Integrated intensity profile of light beam from microprobe for two probes with tip diameters of approximately 0.02 cm and 0.04 cm respectively.*

diameter of 0.005 cm; the main factor causing the transmission loss is attributed to absorption of light by the covering paint. The transmission of light in the microprobe when a filament source is used is at least a factor of five smaller than in the case of the laser indicating the presence of a large backreflection effect.

The main conclusion from these preliminary studies of microprobe design is that reasonably high quality light characteristics of the probe can be obtained if it is used in combination with a laser beam. The parallelity of the exit beam of the probe achieved in this case indicates that in biological material the volume of the sampling being seen by the probe will be approximately ℓd^2 . Here d is the diameter of the probe tip and ℓ is the effective distance of absorption of light in biological material which is usually a few hundred microns for visible light but may be considerable shorter for uv light (3). In a typical situation, therefore, with $\ell = 0.01$ cm and $d = 0.01$ cm the probe will measure fluorescence from a well-localized region with a volume of approximately 10^{-6} cm³.

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**Preliminary Assessment of Fallout from
the 1976-78 Nuclear Weapons Tests
Conducted by the People's Republic of China**

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In September 1976 the People's Republic of China began conducting a series of tests of nuclear weapons. The tests conducted to date have all been carried out at Lop Nor in the Sinkiang Uighur Autonomous Region. The dates, times, and yields of those tests conducted through 1 November 1978 are listed in Table I (1).

TABLE I *Nuclear Weapons Tests '76-'78 People's Republic of China*

DATE	TIME	YIELD
I 26 SEPT 76	0600 GCT	20-200 ktons
II 17 OCT 76	0500 GCT	<20 ktons*
III 17 NOV 76	0600 GCT	4 megatons
IV 18 SEPT 77	0700 GCT	<20 ktons
V 14 MAR 78	0500 GCT	<20 ktons

*underground

Four of the five detonations were in the atmosphere and posed potential hazards to the U.S. population, as well as others, as a consequence of fallout from radioactive debris transported throughout the northern hemisphere by normal weather systems. As an example, the projected path of the cloud from the blast of 17 November 1976 is shown in (Fig. 1) (4). On the occasion of each atmospheric test the Environmental Protection Agency (EPA) activated the Environmental Radiation Ambient Monitoring System to measure possible activity due to several fission fragments, including Sn, La, Ba, Cs, I, Ru, and Ce.

When announcement of the 26 September 1976 test was made in the news media, we decided to evaluate the ability of the Indiana State University Physics Department's gamma coincidence spectrometer to detect fallout from the cloud, should it pass over Terre Haute as predicted. We had available a battery of high volume air samples calibrated to EPA standards (2) and a very sensitive, low-level two-dimensional gamma coincidence spectrometer capable of routine activity measurements in the sub-picocurie range. The spectrometer has been described by Grismore, et al (3).

It was determined that the best opportunity for measuring fallout with the system would be to look for coincidences in the decay of the fission product ¹⁴⁰Ba and its decay products ¹⁴⁰La and ¹⁴⁰Ce. Atmospheric particulate samples were

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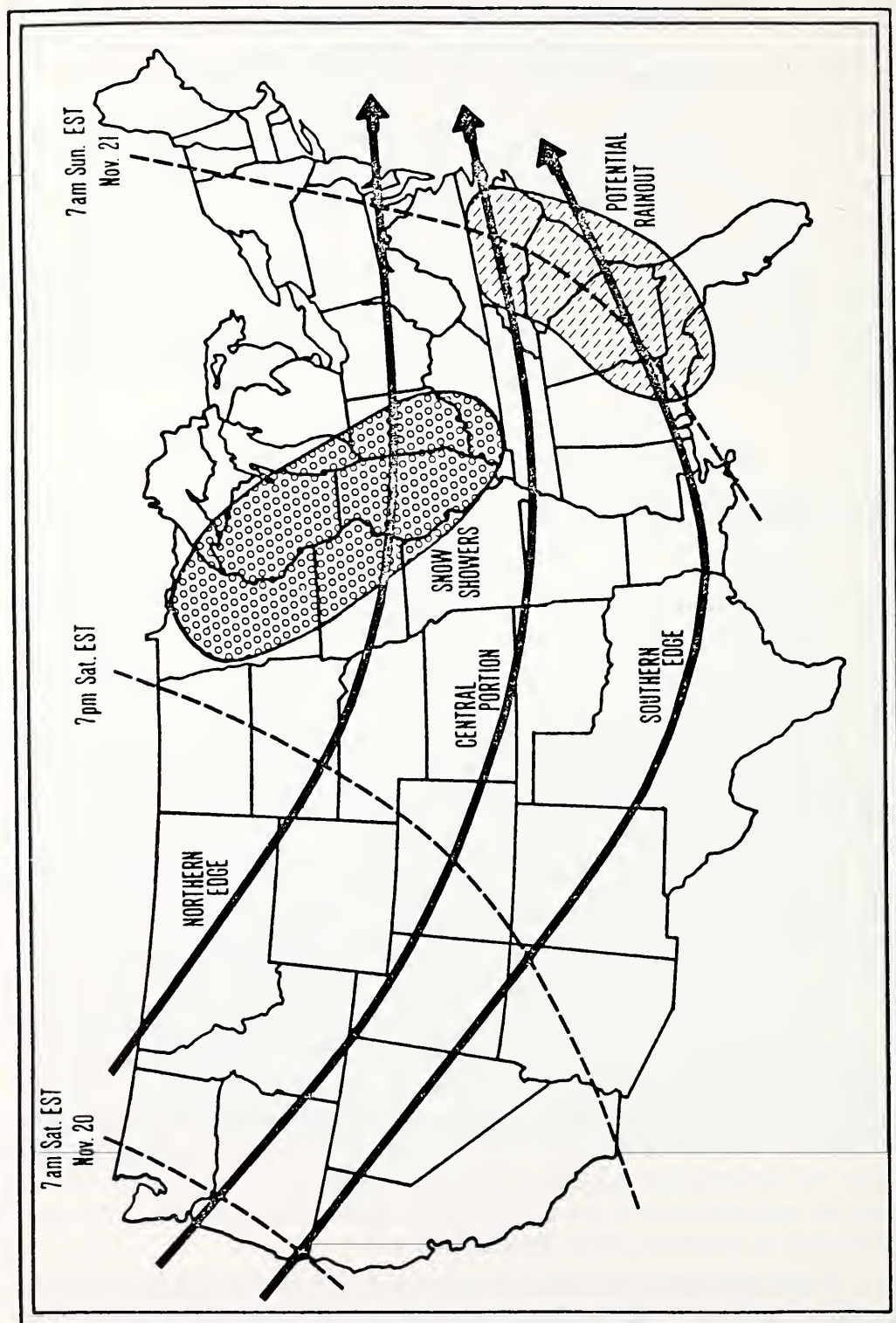


FIGURE 1. Predicted movement of air mass containing radioactive debris across the United States and possible areas of rainout from this air mass following the Chinese nuclear detonation of November 17, 1976.

collected on dried, pre-weighed standard glass fiber filters by high volume samplers located on the roof of the Science Building on the Indiana State University Terre Haute campus. Sample collection in each case was begun within a day or two following the announced time of the test in order to establish baseline background coincidence rates. Sampling was continued for seven to ten days following the initial detection of activity in excess of background. The arrival of the radioactive cloud was quite pronounced as illustrated by the example of test IV shown in (Fig. 2).

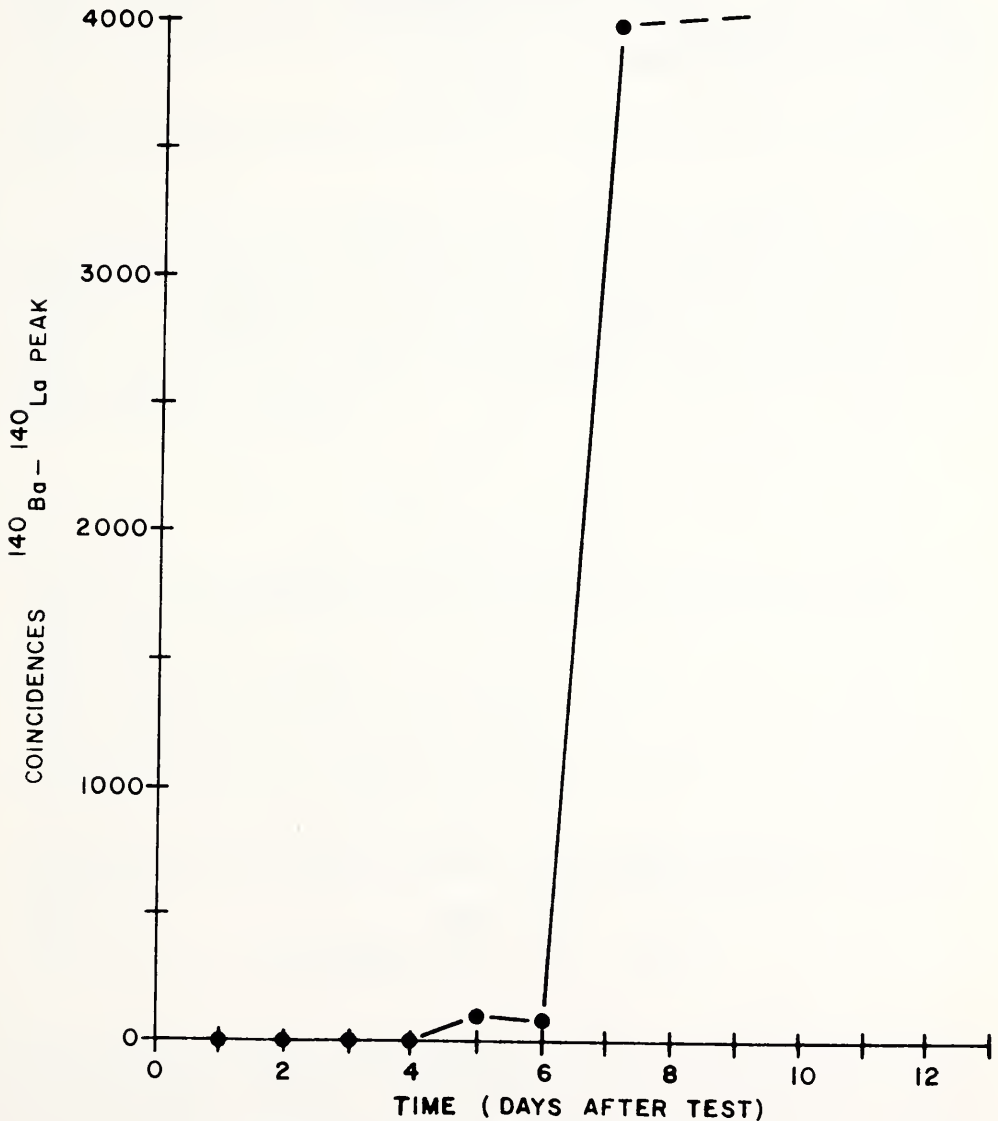


FIGURE 2. *Arrival of Radioactive Cloud Test IV*

The radioactivity collected in the particulate sample was measured by placing the dried, weighed filter into the counting volume and recording coincidences for approximately 22 hours. After background correction,² the

²The coincidence background rate is 6.24 ± 0.04 per 32 kev x 32 kev channel per 24 hours in the energy region of interest.

data were printed out in a two-dimensional array and compared manually with standard coincidence spectra. In the series of experiments reported in this paper analysis was done only for $^{140}\text{Ba} - ^{140}\text{La}$, the decay whose coincidence rate was expected to be most favorable. Standards of $^{140}\text{Ba} - ^{140}\text{La}$ from two different suppliers were used to prepare comparison spectra. To provide additional verification that the coincidence peaks identified with $^{140}\text{Ba} - ^{140}\text{La}$ were really associated with those isotopes, the decay of activity in several of the samples was monitored for about two months with the results as shown in (Fig. 3). The data fit ^{140}Ba very well. Considering the time elapsed since production, it is reasonable to assume that ^{140}La was in equilibrium in the particulate samples collected for these experiments.

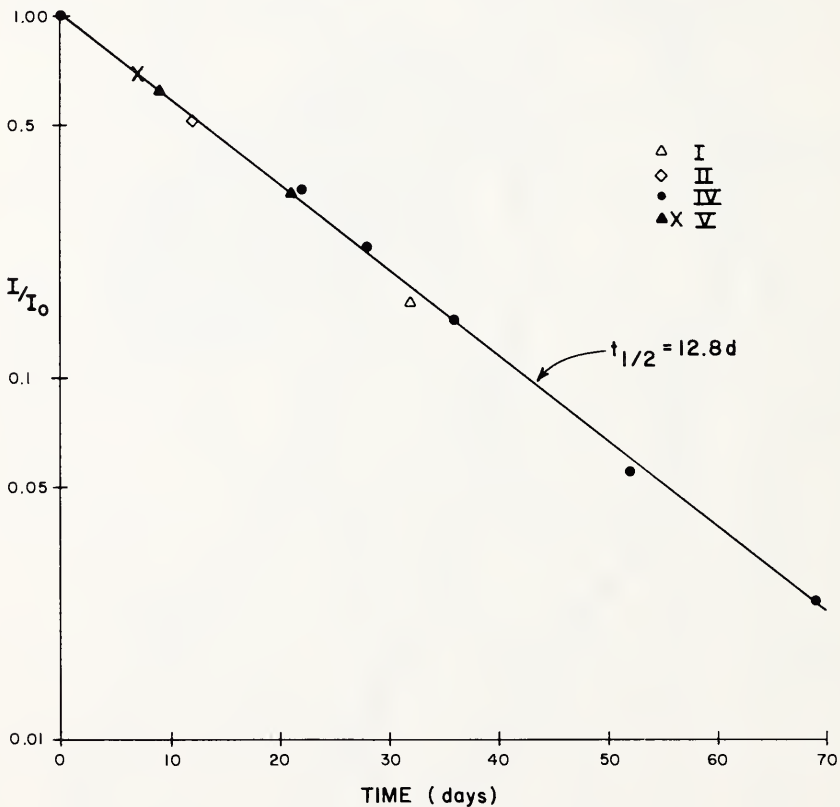


FIGURE 3. Decay of Hi Vol Samples

The concentration C of $^{140}\text{Ba} - ^{140}\text{La}$ in pCi/m^3 of air based on the measured gamma coincidence rate in ^{140}La and its daughter ^{140}Ce is given in Table II for the tests of 18 September 1977 and 14 March 1978. The daily radiation dose ID delivered to individuals in the population by these concentrations were computed from equation 1 below:

$$ID = 24 C (DF) \quad (1)$$

where ID = the individual dose in mrem per day, C = radionuclide concentration in pCi/m^3 of air, 24 = hours in one day, and DF = dose factor for total body submersion in mrem/hr per Ci/m^3 (6).

The total dose received by individuals from this source is determined by integrating the daily contributions over the period of exposure. Preliminary

experimental values determined for ID are also given in Table II for the weapons test of 18 September 1977 and 14 March 1978. The values of ID shown are for exposure to ^{140}Ba - ^{140}La only; they are of the same order of magnitude as those for the weapons tests of 26 September 1976 and 17 November 1976 published recently by Smith, et al (5).

TABLE II *Results*

TEST	MAXIMUM CONCENTRATION	DOSE*
	C (pCi/m ³)	ID (mrem)
IV	0.047 ± 0.002	3.55×10^{-5}
V	0.131 ± 0.005	9.9×10^{-5}

*ID = 24 C (DF)

Contributions to the total dose would also accrue from other radionuclides present in the fallout; e.g. one would expect the contribution from ^{131}I to exceed that from ^{140}Ba - ^{140}La by about a factor of ten. The measured dosages, while significant, are well below currently accepted maximum permissible exposure limits. Analysis of the data to determine the contributions from other radionuclides is continuing.

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PLANT TAXONOMY

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Abstracts

The Identification and Evaluation of Indiana's Rare Vascular Plants: A Preliminary Report. JOHN A. BACONE, Indiana Department of Natural Resources, Division of Nature Preserves, 601 State Office Building, Indianapolis, Indiana 46204. Since Deam's *Flora of Indiana* was published in 1940, many significant changes have taken place with respect to Indiana's flora. The destruction of habitat due to increasing urbanization, industrialization, and agriculture has resulted in many native plant species becoming increasingly rare. At least six species have been extirpated from Indiana, eleven are possibly extirpated, and over thirty species are now known from only one site. It is necessary that these and other species of concern be identified, and their existence verified in the field. The Division of Nature Preserves of the Indiana Department of Natural Resources, and the Biological Survey Committee of the Indiana Academy of Science have been actively working toward this goal, and have prepared a preliminary list. On this list, species are listed as extirpated, possibly extirpated, endangered, threatened, vulnerable, or uncertain. Within the next year, it is hoped that the Division of Nature Preserves, the Biological Survey Committee, and the Indiana Heritage Program can finish herbaria searches, update the nomenclature, and consult the botanists and taxonomists familiar with Indiana's flora, to verify the continued existence of these plants and their abundance. A final list will then be published and periodically updated. The preliminary list will be available at the Indiana Academy of Sciences Fall 1978 meeting.

A Revision of Deam's Trees of Indiana. BARBARA J. HELLENTHAL* and THEODORE J. CROVELLO, Biology Department, University of Notre Dame, Notre Dame, Indiana 46556. A revision of information on the occurrence and distribution of Indiana trees is badly needed due to accelerated human induced environmental changes in the State. To satisfy this need for a reference work we are preparing a hard copy revision of Deam and Shaw's 1953 *Trees of Indiana*. But it also will have several additional features including a request for assistance by citizens around the State and information regarding procedures for their participation. Specifically, their help is needed to: reverify old county level records; help identify new ones; and provide more specific locality information. In our attempt to provide the most recent distribution information and accurate nomenclature for each species, problems have arisen. Many name changes have taken place since the earlier works. Dr. John Kartesz of the Biota North America Committee has thoroughly reviewed and corrected the tree list including

recombination of some varieties. Our keys will identify to the species level, including some species that are found only in cultivation. The text will also describe varieties, forms and hybrids. A map for each species will provide county distribution information as it is presently known. Use of a series of symbols for each county record will provide data on the source of information. In addition to the formal publication of an updated *Trees of Indiana*, a computerized I-TREES (Indiana Trees) data bank is being created at the University of Notre Dame to store current and future information as it is received. This will assure up-to-date information on tree species distributions between future hard copy revisions of *Indiana's Trees* as well as serve as a learning resource for students and other citizens.

Computer Data Bank for Storage of Floristic Data on Swamp Rose and Spicer Lake Nature Preserves. VICTOR L. RIEMEMSCHNEIDER, Department of Biological Sciences, Indiana University at South Bend, South Bend, Indiana 46615._____Field and laboratory notes and measurements made during the past three years in completing a list of vascular plants of the Swamp Rose and Spicer Lake Nature Preserves are stored on computer tape. The data files, labeled SWROSE and SPLAKE, can be accessed by batch or terminal from any computer center in the Indiana University computer network. Each species entry in the files contains the specific location of the specimen, the soil type, habitat type and associated species. In addition, some species entries contain measurements and comments made in the field or in the laboratory while identifying the specimen. Some of the information is in coded form which is explained at the beginning of the file. Computer data banks are a more logical method of communicating massive amounts of information than is publication. They also provide the flexibility of making additions or corrections as new information becomes available. Information concerning file access can be obtained from the author.

The effect of data base choice in quantitative phytogeographic studies. CLIFTON KELLER, University of Notre Dame, Notre Dame, Indiana 46556 and Andrews University._____Numerical taxonomic studies have been shown robust to errors in homology, missing data, measures of similarity, and relatively so to the choice in the number of characters. Subsets of plant distribution records from Indiana were chosen and analyzed by quantitative techniques to determine the effect of this choice on the delimitation of phytogeographic regions. Results from these analyses were compared to the results obtained from an analysis using plant distribution records for all species having maps in Deam (1940) and to published vegetation maps for Indiana. Phytogeographic analyses are shown to be affected by subset selection. Large random subsets and data representing large diversified families provide rough ideas of the relationships among counties. Special results from smaller selected subsets provide additional dimensions into the distribution of particular habitat and community types. Differences among the various results are reasonable when phytogeographic regions are conceived without well defined boundaries.

Computer-Generated Summarization Graphics of the Brassicaceae of the Soviet Union. DOUGLAS C. MILLER* and THEODORE J. CROVELLO, Department of Biology, University of Notre Dame, Notre Dame, Indiana 46556._____Com-

puter-generated summarization graphics provided new insights into the phytogeography of the mustard family (*Brassicaceae*) of the Soviet Union. Plant distribution published in 1939 in Volume VIII of *The Flora Of The U.S.S.R.* was captured for computer retrieval and analysis. This consisted of data on 128 genera and 757 species. An integrated use of custom-written and commercially available programs, in both batch and time-sharing modes, made possible efficient graphic answers to interesting questions in systematics and phytogeography that were previously unanswerable. These methods have produced graphic summaries of the plant topography of geographically restricted species, and may prove of special value for the study of rare and endangered species.

Computerized Data Retrieval at the University of Kansas Herbarium. LARRY A. HAUSER, Department of Biology, University of Notre Dame, Notre Dame, Indiana 46556.——A computerized data retrieval system in use at the University of Kansas Herbarium allows the user quick and easy access to the latest Kansas vascular plant distribution information. It is a dynamic system, in that the data file may be modified and increased as additional systematic studies are completed and new distribution records discovered. The system fills the specific needs of the Herbarium and the closely associated State Biological Survey of Kansas. It is compatible with the Smithsonian-developed SELGEM system already in use at the University of Kansas Natural History Museum. Printouts help botanist in the field make the most efficient use of their time. The present data file includes vascular plant distributions for Kansas by county and also indicates the presence of a KANU voucher specimen. The file is created and edited using the SELGEM system and then most often retrieved by more specific programs. Types of printouts which may be obtained through the system include both county checklists and lists of plants which are not recorded for a particular county but occur in the surrounding area. Among other options available are the printing of dot maps and county summaries. The idea was not to invent yet another retrieval system, but to adapt an available widely used one. Consequently, specific local needs are met while maintaining compatibility with other SELGEM files.

Pollination in *Asarum canadense* and *Aristolochia serpentaria*. CHARLES B. HEISER, Indiana University, Bloomington, Indiana 47401.——Experiments at Bloomington demonstrate that bagged flowers of *Asarum canadense* will set seed thus confirming the observations made by Wildman (*Science* 111:551. 1950), presumably in West Virginia. No insects or other visitors on the flowers were observed during the height of the blooming season, suggesting that autogamy (or possibly apomixis) is the normal mode of seed production in this species. Additional observations should be made, however, for Vogel (*Flora* 167:329-366. 1978) has recently reported that fungus gnats are the pollinators of *Asarum caudatum*. Field experiments with *Aristolochia serpentaria* were unsuccessful. A single plant placed in the greenhouse, however, was observed to set seed in each of two years. It is unlikely that any pollinating agents were present in the greenhouse. Moreover, after the production of chasmogamous flowers, several cleistogamous flowers (see Pfeifer. *Ann. Mo. Bot. Gard.* 53:1-114. 1966) were produced which also set seed. Field studies are required before

concluding that autogamy (or apomixis) is the common method of reproduction in this species.

Phylogenetic significance of pollen morphology in Asiatic species of *Schefflera* (Araliaceae). JANE R. SHOUP* and CHARLES C. TSENG, Purdue University Calumet Campus.——Pollen morphology is used as an indication of phylogenetic relationships among 46 Asiatic species of *Schefflera*. On the basis of several palynological features seven pollen types are distinguished. These types correlate well with other characteristics, particularly number of carpels and geographical distribution of each species. On this basis evolutionary trends in sexine differentiation are postulated. Polymery of floral parts is assumed to be a primitive condition in Araliaceae. Pollen of multicarpellate species, found in New Guinea and the Solomon Islands, is designated *thaumasiantha* type. Pollen in these primitive species is spheroidal, having smooth, imperforate tecta and simple exine structure with no or indistinct bacula. Pollen of about half of the species studied is categorized as *leucescens* type. These species, distributed mainly in Sumatra, the Phillippines, and South China, are mainly pentacarpellate and possess very small pollen grains with relatively undifferentiated sexines similar to the ancestral group. The three Philippine species comprising the *digitata* pollen type have 8-12 carpels and pollen grains with uniformly semitectate sexines and distinct bacula. The pentacarpellate species of the *hoi* type occur in South China. The pollen has tectate-perforate sexines. Two 6-carpellate species from Borneo and Malaya belong to the *calyptata* pollen type; these grains have undulate-foveolate sexine patterns. The *longifrutescens* type is found in a single pentacarpellate Philippine species. This species has small grains with thin exines, short bacula, and suprategal spinules. Another single pentamerous species of New Caledonia is distinguished by the *golip* type pollen in which the sexine is significantly thickened at the poles, suggesting an advanced condition. Proposed phylogenetic relationships are presented. The palynological study of this genus has provided a basis for pollen phylogeny within and without the family Araliaceae.

Edward Lee Greene And His Botanical Collections. MAUREEN A. SAMPSON* and THEODORE J. CROVELLO, Department of Biology, University of Notre Dame, Notre Dame, Indiana 46556.——Edward Lee Green was a noted and controversial figure in plant taxonomy. As one of the earliest taxonomists in the western United States, he made significant contributions to the classifications of many new species. Greene named over 4500 species, including almost 3000 species new to science. Just before his death, Greene left his personal herbarium and library to the University of Notre Dame. The wealth of information stored here is now being studied further. In 1972, Crovello finished the capture for computer retrieval of specimen label data of the 65,000 specimens in Greene's collection. Computer searches are most valuable when they involve questions that are not taxon oriented, but framed primarily in terms of other characters such as geographic location, or individual collectors. Consequently, the computer searched the herbarium data bank for information recorded about Greene's specimens, arranged them chronologically, providing an itinerary for Greene. The information has also been sorted for the compilation of an annotated list of the type specimens in the collection. Statistical and graphic

analysis of these and other aspects of the collection provide insight into the life and works of E. L. Greene and of many contemporaries whose collections are represented in the Greene Herbarium.

Contribution of Seed Protein Studies to the Taxonomy of *Cannabis sativa* (Cannabaceae). CHARLES T. HAMMOND*, Department of Biology, St. Meinrad College, KATHRYN KAMO, and PAUL G. MAHLBERG, Department of Biology, Indiana University, Bloomington, Indiana 47401.——Soluble seed protein profiles of ungerminated achenes of *Cannabis sativa* (marihuana) were compared by SDS acrylamide gel electrophoresis. The achene samples consisted of 23 seed sources of worldwide distribution selected to encompass a range of known intoxicant, semi-intoxicant and nonintoxicant groups. Electrophoretic banding patterns compared visually and by densitometer tracings revealed a highly similar profile in terms of band number, band position, and band density among all seed sources regardless of country of origin or drug type. These data are interpreted to support the concept of a monotypic genus for *Cannabis*.

An analysis of chromatographic patterns of phenolic substances from the foliage of thirteen taxa of American alders (*Alnus*, *Betulaceae*). JOHN J. FURLOW, Department of Biology, Capital University, Columbus, Ohio.——Phenolic compounds were obtained from dried leaves collected from populations throughout the geographical ranges of 13 American taxa of *Alnus*. These substances were extracted in boiling water, ethyl ether, and normal butyl alcohol and separated by two-dimensional descending paper chromatography. Spots were identified on the basis of color reactions, their R_f values calculated, and the resulting patterns analyzed by means of cluster analysis and principal components analysis. A total of 91 compounds were identified in the butanol fractions, from 23 to 52 compounds appearing on the chromatograms of each taxon. At least one unique compound was present in all but four taxa, and all of the taxa were found to be distinguishable on the basis of the patterns observed. Numerical analyses of the results showed several pairs of taxa long considered to be closely related to possess very similar phenolic patterns. These include *Alnus crispa* and *A. sinuata*, *A. rugosa* and *A. tenuifolia*, *A. rugosa* and *A. serrulata*, and *A. arguta* and *A. glabrata*. It is suggested that at least some of these pairs may be conspecific. Close affinity is demonstrated by phenolic patterns but not by morphology between *Alnus rubra* and *A. tenuifolia* and between *A. maritima* and the Latin American species *A. arguta* and *A. jorullensis*. Additional study will be needed to determine the actual relationships among those taxa. Three major clusters of taxa appear in the analyses, these corresponding to subgenera or parts of subgenera. The most remote such cluster is made up of *Alnus crispa* and *A. sinuata* (subgenus *Alnobetula*). The others include *Alnus rugosa*, *A. tenuifolia*, *A. serrulata*, and *A. rubra* on the one hand, and the Latin American group, *A. arguta*, *A. glabrata*, and *A. jorullensis* on the other (each of these clusters representing a relatively distinct part of subgenus *Alnus*).

Techniques Utilized in the Preparation and Analysis of Oak Enzymes. JAMES J. TOBOLSKI, Indiana University-Purdue University at Fort Wayne, Fort Wayne, Indiana 46805.——Markers such as isozymes could be a useful tool in clarifying the taxonomy of the genus *Quercus*. However oak tissue is known to

contain numerous endogenous phenolic compounds and other inhibitors of enzyme activity. The purpose of this investigation was to develop an appropriate enzyme extraction medium and to determine which plant tissues provide suitable quantities of active enzymes. To overcome these endogenous inhibitors several homogenizing buffer systems were tested on bud, cotyledon, epicotyle and leaf tissues of red oak, white oak, swamp white oak and bur oak. The two following homogenizing mediums were found to be equally effective: (1) 0.1 M tris-citrate and lithium borate, pH 6.8; containing 0.2 M diethyldithiocarbamate and 0.1% B-mercaptoethanol, and (2) 0.1 M tris-HCl, pH 8.0 containing 6 mM ascorbate, 6 mM cysteine, 0.5 M sucrose and 1% v/v tween 80. From 0.8 to 1.5 grams of fresh tissue was ground by hand with 5 to 8 drops of homogenizing buffer and 0.8 to 1.5 grams of insoluble polyvinylpolypyrrolidone. The homogenate was centrifuged at 4°C for 10 minutes at 20,000 XG and the supernatant was utilized for enzyme analysis.

The enzymes alcohol dehydrogenase (ADH), leucine aminopeptidase (LAP) and glutamate-oxaloacetate transaminase (GOT) were resolved by starch gel electrophoresis. LAP and ADH isozyme bands were dark and well resolved from bud and cotyledon tissue, light-banded from epicotyle tissue and faint-banded or absent from foliar extracts. GOT was only partially resolved from bud, cotyledon and epicotyle tissues. No GOT bands were recovered from foliage. Isozyme differences between tissues of the same tree were minor. LAP and ADH exhibited a dark staining zone and a light staining zone each with one or two bands. Several species were found to have identical enzyme bands for one or more of the enzymes studied, reflecting their close phylogenetic relationship. However, some isozymes appear to be species specific and could be useful as genetic markers. Several other homogenizing buffers and enzymes are currently being investigated.

The Indiana Biological Survey: An Unending Synthesis

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Introduction

Every year more people become increasingly aware of the high level of interaction and dependence between human activity and the quality of our environment, particularly its living component. This awareness is increasing because plants and animals growing in more or less natural communities satisfy many physical and mental requirements of our lives. But such awareness also is due to a growing moral maturity which includes not only concern for the human species or consideration of primates or other animal species, but also a moral responsibility towards plants and of the general environment itself. Questions traceable to this widening moral awakening have revealed an embarrassing and critical gap in our knowledge of animal and plant species, and of the environments in which they are found. Even more surprisingly, this lack of information is even acute for rare species, which in some ways should be easier to study. We are in the difficult position of knowing what we should do in terms of moral decision-making, but we are unable to act due to lack of sufficient and reliable information!

This paper provides insights into what is being done in Indiana to assemble past information on the State's biota, to make useful summaries of these data available quickly and economically, to point out important gaps in our knowledge, and to increase awareness in all citizens of our natural heritage. These are the tasks of the Indiana Biological Survey. We hope that its approaches and methodologies also can serve as a model for the organization of similar long term surveys in other states. Such studies by their nature and duration, must involve many people and many institutions.

History of the Indiana Biological Survey

The Indiana Biological Survey (IBS) is the main activity of the Biological Survey Committee (BSC), a standing committee of the Indiana Academy of Sciences. The BSC chairman (currently Crovello) and other committee members are appointed by the Academy's President. Formed in 1892, the BSC is almost as old as the Indiana Academy itself. As stated in the 1893 Proceedings of the Academy, its original purpose was, "... 1) to ascertain what has already been accomplished in the direction of making known the character and extent of the life of the State, and to this end to prepare a complete bibliography of materials bearing on the botany, zoology and palentology of Indiana, to be published by the Academy; 2) to associate the various workers throughout the State and so correlate their labors that all will work together towards a definite end, and ultimately accomplish the main purpose of the survey, namely, the making known of the entire fauna and flora of Indiana, its extent, its distribution, its biological relations, and its economic importance; 3) to stimulate the teachers of

biology throughout the State to encourage in their pupils the accumulation of material, which shall make known the local extent and distribution of life forms, and thus contribute facts that will be useful in the survey and at the same time develop acute observers for continuing the study of the natural resources of the State; 4) ultimately to secure for the academy a collection that will illustrate the biology of the State.”

Even though several people (Deam, 1940; Lindsey, 1966; and Lindsey et al, 1969) actively studied and surveyed the State’s biota, over the last several decades BSC activity was confined to an annual survey of the literature.

As indicated above, a growing need for diverse and detailed information on the biota of the State is apparent. However, potential users of such information, no longer restricted to academic biologists, now include such diverse people as preparers of environmental impact statements, employees of State and federal agencies, teachers at all levels, area and regional planners, and individuals with various private interests. More importantly, federal legislation (e.g., the Endangered Species Act of 1973) now requires developers to demonstrate that their activity will not endanger certain species. This and other legislation require a basic, detailed knowledge of the biota of Indiana. It is essential that such knowledge be accumulated, that subsections of it relevant to a specific task be made available in a useful way, and that citizens of the State become aware of their valuable heritage.

As a first step in this direction, in 1974 Crovello proposed to the Academy: “That a formal Flora Indiana Project be created whose major purpose will be the continued maintenance and dissemination of information about the kind, quantity and distribution of the plant life of the State. The information accumulated would be maintained and disseminated in ways that are both useful and efficient.” The motion was adopted by the Academy in 1975 and subsequently formed the basis for an expanded revitalization of the BSC. The current goals of the BSC are the same as when the Academy was founded: to accumulate, store and make available in useful forms, information on the biota of the State.

Given the magnitude of the data base of interest, and the need for fast retrieval of selected pieces of it, we are using computers to help the BSC achieve its goals of accumulating and disseminating information. Since modern society created many of our environmental problems, it is fitting that the computer, an invention of modern society, should contribute to their solution.

Current Activities of the BSC

Given its purposes and building on the previous activities of the BSC in cataloging the literature of the State, (for which we must thank its past Chairman, Jack Munsee), the BSC is providing real or moral sponsorship to the following projects:

A. *BSC Literature Project*—Its purpose is to maintain and to accumulate information on published or semipublished references on the biota of the State, as well as works in progress. While in the past the BSC Literature Project has presented its findings in conventional printed form, we also have begun the

simultaneous creation of an cumulative computer data bank. It can provide updated, intergrated bibliographies of particular topics in response to specific requests. With support from the Academy and from Notre Dame, the following has been accomplished:

A1. I-LIT-OLD: A computerized file of ove 1500 references to the biota of Indiana, obtained by searching the pages of the Proceedings of the Indiana Academy between 1892 and 1976. While most references are from articles in the Proceedings, a number are from other sources that were referred to in the Proceedings. Information on each reference includes: author; date; title; citation; counties studied, (where easily obtainable); and type of article. Given the available resources, this computer file is incomplete. *Please* help us help Indiana by informing us of any omissions that you find. Simply use the latest reporting form, copies of which are available from the BSC.

A2. I-LIT-NEW: Beginning in 1977, an expanded Reporting Form is being used to assemble the recent literature AND works in progress about the biota of Indiana. Beginning with the 1978 literature, we will type completed forms into the computer and simultaneously create our computerized literature file *and* provide manuscript copy to the editor of the Proceedings, without expensive rekeystroking. Naturally, proofreading and error correction will have to be carried out on the computerized file before the computer prints out the manuscript copy. Much more information is being requested than in the past. But readers also will realize that this means they can request computer searches on this much more information. Thus, if one is interested in work on endangered species in Switzerland County, the computer will be able to answer that exact request.

As in the past, Reporting Forms continue to be sent to the chairpersons of the approximately 70 college and university life sciences departments in the State. They are requested to pass along copies of it to members of their departments who either have published on the State's biota or are engaged in research on it. This includes students as well as faculty. Beginning this year the BSC also is distributing Reporting Forms at the annual meeting of the Academy. But we still are not reaching everyone we should reach. *Please* provide the BSC with the name and address of any peson or organization that should receive Reporting Forms on a regular basis (e.g. state agencies, consulting firms).

B. *BSC Endangered Species Project*—Its purpose is to develop and maintain information on the status of species that might be threatened or endangered. Progress is being made in studying several large taxa, including mammals, fish and vascular plants. The BSC cooperates with John Bacone (Division of Nature Preserves, Department of Natural Resources) in the production of a list of rare and endangered vascular plants. Several Academy members (John Whittaker, H. E. McReynolds, and James Gammon) have completed a manuscript on rare vertebrates of the State. Finally, steps have been taken to assure maximum cooperation and mutual enhancement of the work of the BSC and Indiana's Natural Heritage Program. Additional collaborators are always welcome, but especially for nonvascular plants and invertebrates.

C. *BSC Flora Indiana Project (FLIP)*—Currently information is available on the presence or absence of each of approximately 2,500 vascular plants in each of Indiana's 92 counties. The BSC file is based on Deam's (1940) *Flora of Indiana*, plus 8,000 new records as verified by herbarium specimens, or found in recent works such as Swink (1974). This valuable computerized information is available as a byproduct of a Ph.D. thesis by Clifton Keller, a student of Crovello at Notre Dame. While these data deal only with plants, computer programs and other techniques also will be of use to a *Fauna Indiana Project*. Current services include the ability to provide a checklist of vascular plants in a given county (with rare species indicated as such). A *major* effort this year has been to update Deam's nomenclature. Many changes have occurred since the almost forty years when Deam's work was published. We have engaged the services of Doctor John Kartesz of Biota North America to help us. We also are exploring for the best way to integrate information from other sources (environmental impact statements, park checklists, etc.) into the FLIP data bank. Finally, there is need to produce a revision and expansion of Deam's (1940) *Flora of Indiana*. Persons interested in organizing or contributing to such a project should contact the BSC.

D. *BSC Indiana Vascular Plant Atlas Project*—Crovello and Keller are developing a manuscript for an *Atlas of the Vascular Plants of Indiana*. The final hard copy will look very much like the recent *Atlas of Illinois Plants* produced by Mohlenbrock. One difference is that we will produce camera ready copy of species level maps at a much cheaper rate by relying on special computer graphics facilities at Notre Dame. We hesitated to publish an *Atlas* at this time because it will be incomplete. But on the other hand, it will never be complete if we do not publish something to indicate the current status of our knowledge. Consequently, the *Atlas* will be based on information (at the county level) found in Deam's (1940) *Flora of Indiana*, *plus* the 8,000 new herbarium specimens which constitute new county records that were deposited at Indiana University, Bloomington, *plus* Swink's (1974) new information, *plus* possibly information from one or two environmental impact studies which amounted to a survey of the county in which it was located.

The *Atlas* will be produced as quickly and as cheaply as possible, to permit workers in the field to provide additions and other corrections. Different symbols will be used for each county record to indicate its source. Nomenclature will reflect changes since Deam, and at the same time be tied in with Deam's (1940) classic work.

E. *BSC Indiana Trees Project*—Mrs. Barbara Hellenthal and Crovello continue to work on a modification and expansion of Deam and Shaw's (1953) *Trees of Indiana*. This standard reference is out of print, but a more important reason for the current project is the need for a publication both to summarize current knowledge based on distributions in the past and to integrate it with knowledge of *present* distributions. Many workers, including impact assessment personnel, must know whether species are growing on a certain site in Indiana today, not whether they were there ten, twenty, forty, or more years ago. A detailed statement of purposes, possible contents of publications, *and* why and how citizens of the State (including students) should and could become involved

in a resurvey of the Trees of Indiana, was sent to all BSC members in December 1977. A copy of this December 1, 1977 statement, plus a request for opinions and help, was also sent in December 1977 to the State Forester. We believe that cooperation will be beneficial to the State, to the Division of Forestry, and to the goals of the Academy.

F. *BSC Indiana Environmental Impact Statements (I-EIS)*—We have begun discussions with several state and federal agencies on the possibility of creating a computerized file of detailed information on Indiana biota found in I-EIS's. Such data could be useful in many ways, including the updating of distribution records. Naturally, the source of such records would be retained, to permit users to estimate their relative reliability.

G. *BSC Indiana Vegetation Maps Project*—We are in the planning stages of a project to produce several different vegetation maps of Indiana. Each would be based on a different type of information. Given the goals of the Biological Survey Committee, including its function as an advisory group to state agencies as well as others, a map of the potential vegetation would be very valuable. In addition, currently under way are several projects to use satellite data to produce a *current* land use map of the State. Finally, via our Flora Indiana Project, a computerized table of county level presence or absence for 2500 species of vascular plants already exists. These three approaches need to be compared. We plan to request support for: a) the creation of a natural vegetation map de novo; b) the creation of vegetation maps based on species associations that are available at the county level, to see if information on vegetation associations can be obtained via the many intercorrelations that exist when one has data on 2500 species; and c) to compare these two with the latest land use map produced by satellite.

H. *BSC People Power Project*—This project maintains information on persons interested in any or all aspects of the biota of the State, including the accumulation and use of such data. People with interests in the biota of one geographic area immediately come to mind, as do people with interests in one taxon, one group of species, etc. But people of value to the goals of the BSC are not restricted to biologists. The expertise of geographers, geologists and archeologists to name a few, is of great value to the BSC.

Each Survey Project Is An Integrated, Multistage Process

While each project of the Indiana Biological Survey (IBS) can be described in discrete steps, compartmentalization of a more or less continuous process may be misleading (Crovello, 1970). Surveys are unending, multifaceted assessments of the changing state of dynamic natural and human ecosystems. Many of its different activities are carried out simultaneously. Nevertheless, for convenience we describe IBS operations in six stages: 1) planning ; 2) data accumulation 3) data bank deposition; 4) data bank maintenance; 5) data bank output; and 6) publicity. Each step is briefly outlined below.

Stage One: Planning

Planning has been effected mostly by BSC members, many of whom are not just academic biologists but also are active in groups such as The Nature

Conservancy and The Audubon Society. Interaction occurs also with workers and with other interested citizens throughout the State. Essential questions asked during the planning stage include: 1) what are current and future sources of support; 2) what types of data are required; 3) what types of users are expected; 4) what types of people are available to help; 5) what types of output are in demand now and will be in demand in the future.

Stage Two: Data Accumulation

Three substages of data accumulation exist: a) field work; b) subsequent specimen preparation, typed summaries, or publication; and c) data assembly. These substages are recognized because different people frequently are involved with each substage, each can be done at different times, and each involves different procedures. Field information can be stored in written notebooks, on photographic slides, as dried specimens, etc. The final substage, data assembly, frequently is overlooked. Yet it is both essential and time-consuming. Data assembly involves finding proper floras or faunas, the relevant museum specimens, etc., and then extracting from it the *specific* information required for a *particular* task.

Stage Three: Data Bank Deposition

Data bank deposition involves all activities which transfer information from museum specimens, publications, or data sheets into a computer bank. Some data banks are ecologically oriented, others concentrate on species, and others on specimens. So far most IBS data banks are species oriented at the county level. Each type provides different levels of resolution in response to taxonomic, geographic, or ecological questions about the State's biota.

Stage Four: Data Bank Maintenance

Too often in this age of the Computer Revolution we mistakenly think that once data are in a computer, users can obtain *any* type of desired analysis. The computer can only do what its programs have been instructed to do, and they can only operate on data that have been input and continuously updated to remain current. The computer is fast in retrieval, but many other tasks are associated with the creation and maintenance of a data bank about Indiana's biota. Two types of computer files are important. *Data files* contain the total body of information which, when properly searched and analyzed, provide answers to our questions. Such files must be kept current, which involves correction of information in the data bank, addition of new information, and subtraction of information of no use. The *program file* is the second type of computer file. New questions arise at a regular interval and new programs must be written to answer these new, usually more sophisticated questions.

Stage Five: Data Bank Output

The four major types of data bank output are: 1) prose printouts (or publication); 2) tabular results; 3) summary graphics; and 4) summary statistics.

Prose results requested from IBS include computerized printouts of a county checklist of all plant species, as well as county checklists for only rare plant species. Requests for such information come mostly from environmental impact statement workers and park naturalists. Monographers have received prose printouts summarizing the county level distribution of each species in the

family of interest. All such results were easily and cheaply obtained from the IBS computerized data bank. Prose results also are possible from the IBS Literature Bank. Details were provided in an earlier section of this paper.

Tabular results most requested frequently include information on the number of species per county as well as the number of species per family. The scope of tabular results, like Summary Graphics, is limited only by the creativity of the questioner.

Summary graphics may take several forms, the most common of which is the production of State distribution maps at the county level, usually one map for each species. Information from several sources is used to produce such maps. For example, users of distribution maps may want to know whether a county record for a given species is based on Deam's (1940) study, or a more recent one. This can be answered easily if the source of each county distribution record is stored in the computer. A species map at the county level can use one of several symbols to indicate a county record. For example, a D in a county may indicate that that county record was included in Deam. A number such as 73 could indicate that it was not reported in Deam, but that it was first collected in 1973. Finally, a compound such as D 73 might mean that for a specific county it was both recorded by Deam and the *latest* updated reverification was made in 1973.

A second form of graphic summary is the frequency of rare species by phytogeographic or political region. For example, rare plant data workers may be interested in knowing which counties have the highest number of rare plant species. A computer-assisted search of the entire data bank of information on 2,500 species can, in our case, identify those 485 species occurring in five or fewer counties. The computer then can provide an informative three-dimensional summary of these data. (Fig. 1) is an example, summarizing the distribution of rare plant species in Indiana. The height of the "landscape" on the graph represents the number of species in each of Indiana's 92 counties that only occur in five or fewer counties in the State. In (Fig. 1) the observer is looking at the State from the southeast at an elevation high above the surface. The highest peaks on the graph, in the northwest corner, represent approximately 130 rare species per county. Some counties (such as those in the east central part of the State) have no rare species.

Fig. 1 summarizes at a glance much information about the distribution of "rare" plants in Indiana, and is proof that one graph is worth a thousand data points! It presents reviewers unfamiliar with the State's flora a concise summary of the geographic areas that should be evaluated first for endangered species. Correlation of physiographic, climatic and other geographically oriented variables with the distribution of rare plants also is easier.

Summary statistics also are valuable to understand the plants and animals of Indiana. Summary statistics can be provided for each set of taxa—for example, the average number of counties in which each species occurs. Similar statistics can be compiled to answer questions about a particular geographic unit (such as the average and variance of the number of species per county in the State), or for particular ecological units (for example, the average and variance of the number of species in a community). Bivariate and multivariate analyses

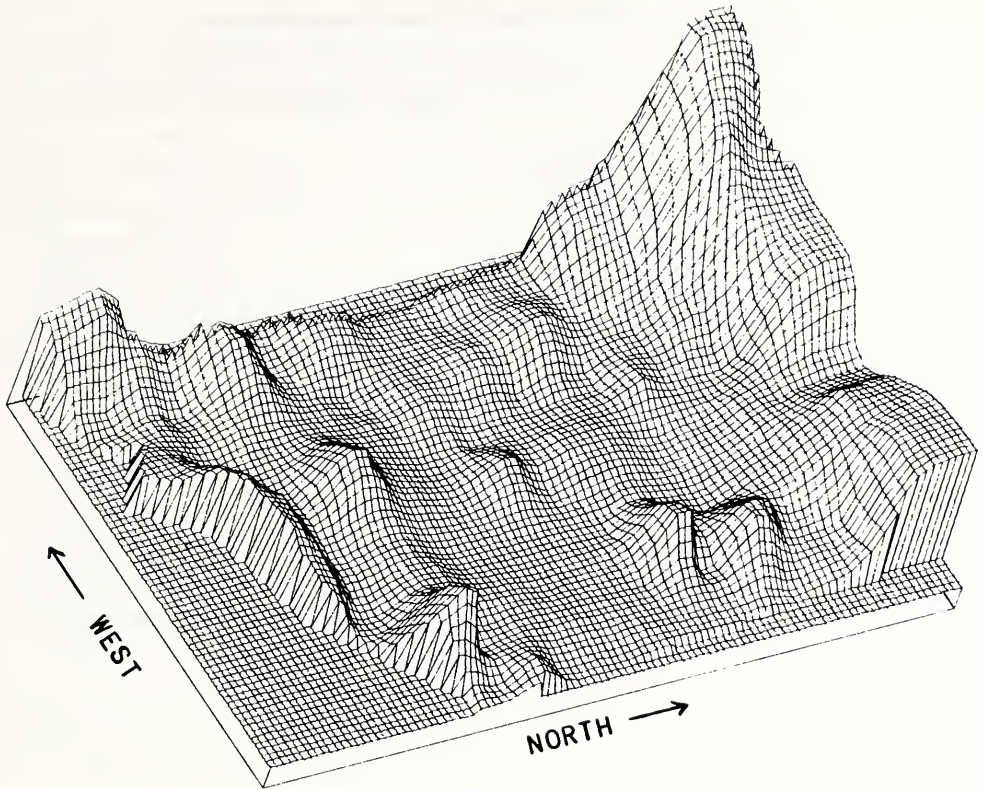


FIGURE 1.

have rarely been used in such studies. The potential is great, and IBS is building on previous experience (e.g., Crovello, 1972; Crovello and Keller, 1974).

To summarize Stage Five, in IBS we believe that conventional publications will always have value for the study of our environment and its inhabitants. But we also believe that computer results also will do much to deepen our understanding of the past, present and future status of Indiana's biota. Finally, in these days of tight budgets, routine computer use also should prove cost effective.

Stage Six: Publicity

The best biological information system is completely useless if potential users are unaware of it. Consequently, every appropriate medium is used to publicize the existence of IBS data banks—This includes the distribution of the minutes of meetings of the BSC, and BSC People Power questionnaires at Indiana Academy of Sciences meetings and by mail as part of the Newsletter of the Indiana Academy of Sciences. Reference is made to IBS data banks in talks on various subjects throughout the country. Publications and fliers explain its existence, availability and use. Members and correspondents of the BSC are urged to establish bulletin boards in their departments that provide information on BSC activity.

Interaction Of IBS With Other Organizations

Following the philosophy that the most valuable and complete work of the BSC will emerge from combined efforts of many people, both from within and outside of the Indiana Academy, continuous efforts are made to involve individuals and organizations throughout the State. To help in planning we have enlisted the aid of informed members of other relevant committees of the Academy, as well as the membership at large. We draw upon State employees, particularly in the Department of Natural Resources, who have provided both information and evaluation of our planned projects. Field workers involved in environmental impact statements who contact us for information are also asked to outline their views on the most urgent information needs for Indiana plant, animal and environmental data.

In data accumulation, we have used printed matter already published by various sources, including the Department of Natural Resources and The Ford Foundation. We are cooperating with the Indiana Natural Heritage Program. Similarly, conservation groups, such as The Audubon Society and The Issac Walton League, include many members knowledgeable in plants and animals. Many advantages will accrue if the lands of such organizations will be inventoried early. Finally, particularly with the woody plant species, we expect that the 4-H Club organization, which is partially funded in every State by the federal government, can be utilized as a source of taxonomic, geographic and ecological information.

Perhaps the largest interaction with other organizations and individuals will be in the area of data bank output. If handled correctly, the IBS data banks can serve many diverse organizations and individuals, including other committees such as the Science and Society Committee of the Indiana Academy, its individual members, and Department of Natural Resources personnel. It could serve as a data base for information on woody plants for such groups as the 4-H Club, Boy Scouts, or Girl Scouts. We plan to explore interactions with the State's Department of Public Instruction, both at the elementary and high school level. The Environmental Protection Agency and other federal organizations will or should find geographical, taxonomic, and environmental information on the plants and animals of the State of great value for many of their projects. National organizations such as Sigma Xi, the American Association for the Advancement of Science, and the American Institute of Biological Sciences, all have science and society committees. Once IBS is begun, it could be a routine task to provide such organizations with information of interest regarding species in Indiana.

Summary

To summarize, we may say simply that IBS is alive, growing, and dedicated to making Indiana and its people better. The long range goal of the BSC is to accumulate, maintain and disseminate information about the kind, quantity, and distribution of the biota of the State. Our philosophical point of view has always been that such surveys are an unending, multifaceted task (Constance, 1964). This task requires many people but it promises both tangible and intangible rewards to both participants and to society in general. IBS is a very

time consuming process, which time could have been spent in other activities. Nevertheless, we believe in the need for IBS and that in the long run it will serve both academic goals as well as those of Society.

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New Records of Plant Species for Switzerland County, Indiana

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Introduction

Quantitative and qualitative sampling of a study area in southeastern Indiana uncovered some significant additions to the recorded flora of Switzerland County. Six distinct vegetation types were studied over the period of 1 year. Many species were found to be restricted to one type, possessing suitable growth characteristics. This presentation of new plant records for Switzerland County, Indiana is directed toward updating and adding to the local and regional flora.

Study Area

The vegetation study area is located in Switzerland County, Indiana which forms a portion of the southeastern corner of the state. Switzerland County falls within the Western Mesophytic Forest Region and in particular, the area of Illinoian glaciation (2). The study site occupies 883 acres and is located along the eastern edge of the county, bordered to the east by the Ohio River. Located in T1N, R1E, Sec. 5 and 6, and T2N, R1E, Sec. 31, the western boundary of the study site borders the First Principal Meridian. The town of Patriot, Indiana is located approximately 4 miles south of the site. On site, topographic relief varies from an elevation of 455 feet (138.6 meters) to 580 feet (176.78 meters) above mean sea level (m.s.l.). First and second bottoms within the Ohio River floodplain predominate on the site, with moderately steep hillside lands found in the western portion of the site. The soils supporting the vegetation of the study site consist of two major types, soils of the first bottoms and soils of the second bottoms (9) (12). The concept of the representative habitat (vegetation type) was employed throughout the field survey. Utilizing this concept, both quantitative and qualitative data were obtained for the vegetation types found on the site. The actual onsite cover types included upland woods, grazed woods, advanced oldfield, oldfield, riparian, and riverbank.

Methods and Materials

The overstory and understory vegetation sampling within the forested vegetation types of the site was accomplished utilizing the point-centered quarter (PCQ) method (4) (13). This method provides quantitative information concerning relative density, frequency, dominance, species frequency and total basal area.

Ground level vegetation was studied using the microplot-quadrat method (5) (11). Additional methods employed included the 1/10 acre area plot used in the advanced oldfield, and increment core analysis used in determining

successional trends. All of these methods coupled with observations and records taken in the field provided input to a detailed analysis of the study site.

To conduct a comprehensive study, reference maps of the county were obtained and evaluated. Several regional vegetation maps were used to categorize the major stand type (2) (7). Taxonomic sources consulted provided a rich source of information including Deam (6), Braun (1), Britton and Brown (3), and Gleason and Cronquist (8). Deam (6) was considered to be the most applicable to the vegetation of the southeastern Indiana study site.

The upland woods sampling location was situated in the extreme western portion of the study site and was found on well-drained ridges, hilltops and upslopes. The grazed woods sampling location was in the western portion of the site. A pronounced browse line was observed along the outer perimeter of this woods. The major portion of this sampling location consisted of dense woods with thick understory and ground cover vegetation. The vegetation of the grazed woods location included both mesophytic and xerophytic species with specific occurrence related to degree of slope.

The advanced oldfield sampling location was within the western portion of the site. It was at a successional stage between incipient oldfield and wooded vegetation types. This location had no true overstory; instead it contained a developing woody understory. The oldfield sampling area was located in the west central portion of the site amidst cropland and pastureland. This oldfield was estimated to be 7 to 10 years old and in the past was subject to light, incidental grazing.

The riparian study area followed a small creek in the northern portion of the site. This community had a sparse understory and overstory vegetation. The riverbank sampling consisted of a narrow ribbon of hydrophytic vegetation adjacent and parallel to the Ohio River. Agricultural lands on site including crop and pastureland were not quantitatively sampled due to the intensive cultivation practices used on site.

Results and Discussion

Table I includes 162 plant species identified from the study area which had not previously been reported for Switzerland County (10). The listed species were recorded and tabulated on the basis of the six predominant vegetation types of the site. In addition, the season and stratum in which the species were observed are recorded. Throughout the seasonal field study, a total of 253 plant species, representative of 189 genera and 75 families recorded. Of the total recorded species, the 162 species presented here, had not previously been reported for Switzerland County. It is probable that the number of new records presented here is not reflective of an influx of new species, but rather represents new records for a county in which there has been a paucity of previous studies.

Switzerland County possesses a recorded flora of 337 plant species (10). These additional records should expand the flora of Switzerland County to 501 taxa.

TABLE I New vegetation species of Switzerland County study site

Scientific Name Common Name	Sampling Area (Upland Woods Vegetation Type)	Sampling Area (Grazed Woods Vegetation Type)	Sampling Area (Advanced Oldfield Vegetation Type)	Sampling Area (Oldfield Vegetation Type)	Sampling Area (Riparian Vegetation Type)	Sampling Area (Riverbank Vegetation Type)
<i>Abutilon theophrasti</i> Medic.						
Velvet-leaf	—	—	C, D	C, D	—	—
<i>Adiantum pedatum</i> L.						
Maidenhair fern	E	—	—	—	—	—
<i>Aesculus octandra</i> Marsh.						
Yellow buckeye	—	—	—	—	—	E
<i>Agastache nepetoides</i> (L.) Ktze.						
Giant hyssop	—	—	—	—	—	E
<i>Agrimonia parviflora</i> Ait.						
Smallflower agrimony	—	—	—	—	E	—
<i>Allium canadense</i> L.						
Meadow garlic	C	—	—	—	—	E
<i>Allium stellatum</i> Ker.						
Wild onion	—	—	—	—	—	D
<i>Alnus serrulata</i> (Ait.) Willd.						
Smooth alder	—	—	—	—	E	—
<i>Ambrosia bidentata</i> Michx.						
Lanceleaf ragweed	—	—	—	C	—	—
<i>Ambrosia trifida</i> L.						
Great ragweed	—	—	C	—	E	E
<i>Arisaema triphyllum</i> (L.) Schott.						
Jack-in-the-pulpit	E	—	—	—	—	—
<i>Artemisia absinthium</i> L.						
Common wormwood	—	—	—	—	—	E
<i>Aruncus dioicus</i> (Walt.) Fern.						
Common goatsbeard	—	—	—	E	—	—
<i>Asarum reflexum</i> Bickn.						
Curly wildginger	—	E	—	—	—	—

TABLE I New vegetation species of Switzerland County study site (Continued)

Scientific Name Common Name	Sampling Area (Upland Woods Vegetation Type)	Sampling Area (Grazed Woods Vegetation Type)	Sampling Area (Advanced Oldfield Vegetation Type)	Sampling Area (Oldfield Vegetation Type)	Sampling Area (Riparian Vegetation Type)	Sampling Area (Riverbank Vegetation Type)
<i>Asclepias variegata</i> L. White milkweed	—	—	—	D	D	D
<i>Asplenium platyneuron</i> (L.) Oakes	E	—	—	—	C	—
Ebony spleenwort	—	—	—	E	—	—
<i>Aster novae-angliae</i> L. New England aster	—	—	—	—	—	—
<i>Avena fatua</i> L. Wild oat	—	—	—	—	D	—
<i>Avena sativa</i> L. Oat	—	—	—	—	—	E
<i>Betula nigra</i> L. River birch	—	—	—	—	A	A, B
<i>Bidens bipinnata</i> L. Spanish needles	—	—	—	—	—	C
<i>Bidens frondosa</i> L. Bidens	C	C	—	—	—	—
<i>Bromus latiglumis</i> (Shear) Hitchc. Bromegrass	—	—	—	—	—	E
<i>Bromus tectorum</i> L. Downy chess	—	—	—	E	—	—
<i>Campsis radicans</i> (L.) Seem. Trumpet-creeper	C	—	—	D	D	C, D
<i>Carduus nutans</i> L. Musk thistle	—	—	—	E	—	—
<i>Carum carvi</i> L. Caraway	—	—	—	—	—	E
<i>Carya ovaia</i> (Mill.) K. Koch Shagbark hickory	A, B	A, B	E	—	—	—

TABLE 1 New vegetation species of Switzerland County study site (Continued)

Scientific Name Common Name	Sampling Area (Upland Woods Vegetation Type)	Sampling Area (Grazed Woods Vegetation Type)	Sampling Area (Advanced Oldfield Vegetation Type)	Sampling Area (Oldfield Vegetation Type)	Sampling Area (Riparian Vegetation Type)	Sampling Area (Riverbank Vegetation Type)
<i>Carya tomentosa</i> (Lam.) Nutt. Mockernut	A, B	A, B	—	—	—	—
<i>Celtis occidentalis</i> L. Hackberry	A, B	A, B	—	—	A, B	A, B
<i>Chenopodium album</i> L. Pigweed	—	—	C, D	—	—	E
<i>Chrysanthemum leucanthemum</i> L. var <i>pinnatifidum</i> Lecoq & LaMotte Oxeye daisy	—	—	C, D	C, D	—	E
<i>Cichorium intybus</i> L. Chicory	—	—	—	E	—	—
<i>Cirsium discolor</i> (Muhl.) Spreng. Field thistle	—	—	—	—	E	—
<i>Cirsium vulgare</i> (Savi) Airy-Shaw Bull thistle	—	—	—	E	—	—
<i>Clematis virginiana</i> L. Virgins-bower	—	—	—	E	—	—
<i>Commelina communis</i> L. Common dayflower	—	—	—	E	—	—
<i>Convolvulus arvensis</i> L. Field bindweed	—	—	—	C	—	D
<i>Convolvulus sepium</i> L. Hedge bindweed	—	—	C	—	—	C, D
<i>Coreopsis major</i> Walt. Large coreopsis	—	—	—	D	—	—
<i>Cornus florida</i> L. Flowering dogwood	—	—	—	—	B	—

TABLE I New vegetation species of Switzerland County study site (Continued)

Scientific Name Common Name	Sampling Area (Upland Woods Vegetation Type)	Sampling Area (Grazed Woods Vegetation Type)	Sampling Area (Advanced Oldfield Vegetation Type)	Sampling Area (Oldfield Vegetation Type)	Sampling Area (Riparian Vegetation Type)	Sampling Area (Riverbank Vegetation Type)
<i>Cynodon dactylon</i> (L.) Pers.						
Bermuda grass	—	—	—	E	—	—
<i>Cyperus strigosus</i> L.						
Umbrella sedge	—	—	—	—	—	E
<i>Datura stramonium</i> L.						
Jimson-weed	—	—	—	—	E	—
<i>Delphinium ajacis</i> L.						
Rocket larkspur	E	—	—	—	—	—
<i>Desmanthus illinoensis</i> (Michx.) MacM.						
Illinois mimosa	—	—	—	—	—	E
<i>Dianthus armeria</i> L.						
Deftford pink	—	—	—	E	—	—
<i>Dicentra canadensis</i> (Goldie) Walp.						
Squirrel corn	D	D	—	—	—	—
<i>Dicentra cucullaria</i> (L.) Bernh.						
Dutchman's-breeches	E	—	—	—	—	—
<i>Diervilla lonicera</i> Mill.						
Bush-honeysuckle	—	—	—	D	E	—
<i>Digitaria ischaemum</i> (Schreb.) Muhl.						
Smooth crabgrass	—	—	E	E	—	—
<i>Dioscorea villosa</i> L.						
Wild yam-root	E	—	—	—	—	—
<i>Dipsacus sylvestris</i> Huds.						
Common teasel	—	—	C	E	—	—
<i>Echinochloa crusgalli</i> (L.) Beauv.						
Barnyard grass	—	—	—	—	—	E
<i>Elymus canadensis</i> L.						
Canada wild-rye	—	—	C	C	—	C

TABLE I New vegetation species of Switzerland County study site (Continued)

Scientific Name Common Name	Sampling Area (Upland Woods Vegetation Type)	Sampling Area (Grazed Woods Vegetation Type)	Sampling Area (Advanced Oldfield Vegetation Type)	Sampling Area (Oldfield Vegetation Type)	Sampling Area (Riparian Vegetation Type)	Sampling Area (Riverbank Vegetation Type)
<i>Eryngium yuccaefolium</i> Michx. Button-snakeroot	—	—	—	E	—	—
<i>Erythronium americanum</i> Ker. Common trout lily	—	D	—	—	—	—
<i>Euonymus atropurpureus</i> Jacq. Wahoo	—	—	—	—	—	E
<i>Eupatorium fistulosum</i> Barratt. Joe-pye-weed	—	—	E	D, E	—	—
<i>Euphorbia corollata</i> L. Flowering spurge	—	—	—	—	—	D
<i>Festuca elatior</i> L. Meadow fescue	C, D	—	C, D	C	C, D	D
<i>Fragaria virginiana</i> Duchesne. Virginia strawberry	C, D	C, D	C, D	C	—	D
<i>Fraxinus lanceolata</i> Borkh. Green ash	—	A	—	—	A, B	—
<i>Geum canadense</i> Jacq. White avens	C, D	C, D	—	C, D	—	D
Soy-bean	—	—	—	—	—	C
<i>Helopsis helianthoides</i> (L.) Sweet Sunflower heliopsis	—	—	—	E	—	—
<i>Ipomoea hederacea</i> Jacq. Ivyleaf morning-glory	—	—	—	—	E	—
<i>Ipomoea purpurea</i> (L.) Roth Common morning-glory	—	—	—	—	—	E
<i>Jeffersonia diphylla</i> (L.) Pers. Twinleaf	—	D	—	—	—	—

TABLE I New vegetation species of Switzerland County study site (Continued)

Scientific Name Common Name	Sampling Area (Upland Woods Vegetation Type)	Sampling Area (Grazed Woods Vegetation Type)	Sampling Area (Advanced Oldfield Vegetation Type)	Sampling Area (Oldfield Vegetation Type)	Sampling Area (Riparian Vegetation Type)	Sampling Area (Riverbank Vegetation Type)
<i>Lactuca canadensis</i> L. var. <i>latifolia</i> O. Ktze						
Wild lettuce	—	—	D	C, D	—	D
<i>Lactuca scariola</i> L.						
Prickly lettuce	—	—	—	—	—	E
<i>Laportea canadensis</i> (L.) Gaud.						
Canada nettle	C, D	C, D	—	—	D	—
<i>Lepidium densiflorum</i> Schrad.						
Common pepperwort	—	—	—	—	—	D
<i>Lepidium virginicum</i> L. var. <i>typicum</i> C. L. Hitchcock						
Peppergrass	—	—	—	E	—	—
<i>Lespedeza cuneata</i> (Dumont) G. Don.						
Silky lespedeza	—	—	—	—	—	E
<i>Lespedeza repens</i> (L.) Bart.						
Creeping bushclover	—	—	—	—	E	—
<i>Ligustrum vulgare</i> L.						
European privet	D	—	—	—	—	—
<i>Linaria vulgaris</i> Hill.						
Common toadflax	—	—	—	E	C	—
<i>Lindera benzoin</i> (L.) Blume.						
Spice bush	B	B	—	—	B	—
<i>Lonicera japonica</i> Thunb.						
Japanese honeysuckle	C, D	C	—	—	—	E
<i>Lycopodium flabelliforme</i> (Fern) Blanchard						
Clubmoss	C	—	—	—	—	—
<i>Maclura pomifera</i> (Raf.) Schneid.						
Osage-orange	—	—	E	—	—	A, B
<i>Malus coronaria</i> (L.) Mill.						
Wild sweet crab	—	—	—	—	A, B	—

TABLE I New vegetation species of Switzerland County study site (Continued)

Scientific Name Common Name	Sampling Area (Upland Woods Vegetation Type)	Sampling Area (Grazed Woods Vegetation Type)	Sampling Area (Advanced Oldfield Vegetation Type)	Sampling Area (Oldfield Vegetation Type)	Sampling Area (Riparian Vegetation Type)	Sampling Area (Riverbank Vegetation Type)
<i>Malva moschata</i> L. Musk mallow	—	—	—	—	E	—
<i>Malva neglecta</i> Wallr. Cheeseweed	—	—	—	—	D	—
<i>Matricaria matricarioides</i> (Less.) Porter Rayless camomile	—	—	—	—	E	—
<i>Medicago lupulina</i> L. Black medic	—	—	—	E	—	—
<i>Medicago sativa</i> L. Alfalfa	—	—	E	E	—	—
<i>Melilotus officinalis</i> (L.) Lam. Yellow sweetclover	—	—	C, D	—	—	—
<i>Menispermum canadense</i> L. Common moonseed	E	—	—	—	—	—
<i>Mentha arvensis</i> L. Field mint	—	—	—	—	C	—
<i>Morus rubra</i> L. Red mulberry	A, B	B	E	—	—	B
<i>Muhlenbergia schreberi</i> J. F. Gmel. Nimblewill	—	—	—	—	—	D
<i>Ornithogalum umbellatum</i> L. Common star-of-Bethlehem	—	—	—	—	—	E
<i>Ostrya virginiana</i> (Mill.) K. Koch Hop-hornbeam	A, B	A, B	—	—	—	—
<i>Oxalis europaea</i> Jordan Lady's sorrel	—	C, D	C, D	C, D	C, D	C, D
<i>Parthenocissus quinquefolia</i> (L.) Planch. Virginia creeper	C, D	C, D	E	C, D	C, D	D

TABLE I *New vegetation species of Switzerland County study site (Continued)*

Scientific Name Common Name	Sampling Area (Upland Woods Vegetation Type)	Sampling Area (Grazed Woods Vegetation Type)	Sampling Area (Advanced Oldfield Vegetation Type)	Sampling Area (Oldfield Vegetation Type)	Sampling Area (Riparian Vegetation Type)	Sampling Area (Riverbank Vegetation Type)
<i>Paspalum pubescens</i> Muhl.						
Paspalum	—	—	—	C	—	—
<i>Passiflora incarnata</i> L.						
Maypop	—	—	—	—	—	E
<i>Phlox maculata</i> L.						
Sweet William phlox	E	—	—	—	—	—
<i>Plantago lanceolata</i> L.						
Buckhorn	—	—	C, D	C, D	D	C, D
<i>Platanus occidentalis</i> L.						
American planetree	A	—	B, E	E	A, B	E
<i>Poa pratensis</i> L.						
Kentucky bluegrass	C, D	C, D	C, D	C, D	C, D	C, D
<i>Polygonatum biflorum</i> (Walt.) Ell.						
Smooth Solomon's-seal	D	D	—	—	—	—
<i>Polygonum pennsylvanicum</i> L. var. <i>genuinum</i> Fern.						
Pennsylvania smartweed	—	—	—	—	C, D	—
<i>Populus deltoides</i> Michx.						
Cottonwood	—	—	—	—	—	E
<i>Portulaca oleracea</i> L.						
Common purslane	—	—	C, D	—	—	—
<i>Potamogeton diversifolius</i> Raf.						
Pondweed	—	—	—	—	E	—
<i>Potentilla norvegica</i> L.						
Cinquefoil	—	—	—	D	D	D
<i>Prunella vulgaris</i> L. var. <i>lanceolata</i> (Bart.) Fern						
American selfheal	—	—	C, D	D	—	D

TABLE I New vegetation species of Switzerland County study site (Continued)

Scientific Name Common Name	Sampling Area (Upland Woods Vegetation Type)	Sampling Area (Grazed Woods Vegetation Type)	Sampling Area (Advanced Oldfield Vegetation Type)	Sampling Area (Oldfield Vegetation Type)	Sampling Area (Riparian Vegetation Type)	Sampling Area (Riverbank Vegetation Type)
<i>Quercus alba</i> L. White oak	A	A	—	—	—	—
<i>Quercus borealis</i> Michx. var. <i>maxima</i> (Marsh) Ashe.						
Red oak	A, B	A, B	E	—	—	—
<i>Quercus coccinea</i> Muench. Scarlet oak	A	A	—	—	—	—
<i>Quercus prinus</i> L. Swamp chestnut	—	—	—	—	—	E
<i>Quercus velutina</i> Lam. Black oak	—	A	—	—	A	—
<i>Raphanus raphanistrum</i> L. Wild radish	—	—	—	—	—	E
<i>Robinia pseudoacacia</i> L. Black locust	—	A, B	—	—	A, B	A, B
<i>Rosa multiflora</i> Thunb. Japanese rose	—	—	C, E	E	—	—
<i>Rosa carolina</i> L. Pasture rose	—	B	—	—	—	—
<i>Rubus allegheniensis</i> Porter Allegheny blackberry	—	—	E	C, D	—	E
<i>Rubus flagellaris</i> Willd. Northern dewberry	—	—	—	E	—	—
<i>Rubus occidentalis</i> L. Common blackcap raspberry	—	—	—	E	—	—
<i>Rudbeckia hirta</i> L. Black-eyed susan	—	—	—	C	—	—

TABLE I New vegetation species of Switzerland County study site (Continued)

Scientific Name Common Name	Sampling Area (Upland Woods Vegetation Type)	Sampling Area (Grazed Woods Vegetation Type)	Sampling Area (Advanced Oldfield Vegetation Type)	Sampling Area (Oldfield Vegetation Type)	Sampling Area (Riparian Vegetation Type)	Sampling Area (Riverbank Vegetation Type)
<i>Rumex crispus</i> L.	—	—	C, D	C	D	C, D
Curly dock	—	—	—	—	—	—
<i>Salix humilis</i> Marsh	—	—	—	—	A	—
Prairie willow	—	—	—	—	—	—
<i>Salsola kali</i> var. <i>tenuiflora</i> G.F.W. Mey.	—	—	—	D	—	—
Russian thistle	—	—	—	—	—	—
<i>Sanguinaria canadensis</i> L.	—	—	—	—	—	—
Bloodroot	E	—	—	—	—	—
<i>Saxifraga virginensis</i> Michx.	—	—	—	—	—	—
Virginia saxifrage	—	—	—	—	C	—
<i>Scirpus validus</i> Vahl.	—	—	—	—	—	—
Bulrush	—	—	—	—	E	—
<i>Secale cereale</i> L.	—	—	—	—	—	—
Rye	—	—	—	—	—	D
<i>Silene cucubalus</i> Wibel.	—	—	—	—	—	—
Bladder catchfly	—	—	—	—	—	E
<i>Silphium perfoliatum</i> L.	—	—	—	—	—	—
Cup rosinweed	—	—	—	E	—	—
<i>Silphium trifoliatum</i> L.	—	—	—	—	—	—
Whorled rosinweed	—	—	—	—	E	—
<i>Smilax glauca</i> Walt. var. <i>genuina</i> Blake.	—	—	—	—	—	—
Sawbrier	—	C	—	—	—	—
<i>Smilax herbacea</i> L.	—	—	—	—	—	—
Smooth carrion-flower	E	—	—	—	—	—
<i>Solanum carolinense</i> L.	—	—	—	—	—	—
Horse nettle	—	—	—	C	—	D
<i>Solanum nigrum</i> L.	—	—	—	—	—	—
Common nightshade	—	—	—	—	—	E

TABLE I New vegetation species of Switzerland County study site (Continued)

Scientific Name Common Name	Sampling Area (Upland Woods Vegetation Type)	Sampling Area (Grazed Woods Vegetation Type)	Sampling Area (Advanced Oldfield Vegetation Type)	Sampling Area (Oldfield Vegetation Type)	Sampling Area (Riparian Vegetation Type)	Sampling Area (Riverbank Vegetation Type)
<i>Solidago latifolia</i> L.	—	—	C, D	C	D	E
Broadleaf goldenrod						
<i>Sorghastrum nutans</i> (L.) Nash.	—	—	—	—	—	E
Indian grass						
<i>Sorghum halepense</i> (L.) Pers.	—	—	—	—	D	D
Johnson grass						
<i>Spergula arvensis</i> L.	—	—	—	E	—	—
Spurrey						
<i>Strophostyles umbellata</i> (Muhl.) Britt.	—	—	—	E	—	—
Trailing wild bean						
<i>Syringa vulgaris</i> L.	—	—	—	—	—	—
Common lilac		B	E	—	—	—
<i>Tanacetum vulgare</i> L.	—	—	—	—	—	—
Common tansy				D	—	—
<i>Taraxacum officinale</i> Weber						
Common dandelion	—	D	D	C, D	—	C, D
<i>Thalictrum dioicum</i> L.						
Early meadowrue	—	E	—	—	—	—
<i>Tradescantia virginiana</i> L.						
Virginia spiderwort	E	—	—	—	—	—
<i>Trifolium arvense</i> L.	—	—	—	D	—	—
Rabbit foot clover						
<i>Trifolium pratense</i> L.	—	—	—	—	—	—
Red clover						
<i>Trifolium procumbens</i> L.	—	—	C, D	C, D	—	D, E
Low hop clover						
<i>Trifolium repens</i> L.	—	—	—	E	—	—
White clover	—	—	D	C, D	—	C, D

TABLE I New vegetation species of Switzerland County study site (Continued)

Scientific Name Common Name	Sampling Area (Upland Woods Vegetation Type)	Sampling Area (Grazed Woods Vegetation Type)	Sampling Area (Advanced Oldfield Vegetation Type)	Sampling Area (Oldfield Vegetation Type)	Sampling Area (Riparian Vegetation Type)	Sampling Area (Riverbank Vegetation Type)
<i>Triodanis perfoliata</i> (L.) Nieuwl.	—	—	—	—	—	E
Venus' looking glass	—	—	—	—	—	—
<i>Trillium erectum</i> L.	E	—	—	—	—	—
Trillium	—	—	—	—	—	—
<i>Typha latifolia</i> L.	—	—	—	—	D	—
Common cattail	—	—	—	—	—	—
<i>Ulmus rubra</i> Muhl.	—	—	—	—	—	—
Slippery elm	B	A	—	—	A, B	A, B
<i>Verbascum blattaria</i> L.	—	—	—	E	—	—
Moth mullein	—	—	—	—	—	—
<i>Vernonia altissima</i> Nutt.	—	—	—	—	—	—
Tall ironweed	—	—	C	C, D	—	—
<i>Viola papilionacea</i> Pursh.	—	—	—	—	—	—
Butterfly violet	C, D	D	C, D	—	D	C, D
<i>Vitis aestivalis</i> Michx.	—	—	—	—	—	—
Summer grape	D	B	—	C	B, E	E
<i>Vitis riparia</i> Michx.	—	—	—	—	—	—
Riverbank grape	—	—	—	—	—	B
<i>Xanthium spinosum</i> L.	—	—	—	—	—	—
Spiny cocklebur	—	—	—	E	—	—
<i>Zanthoxylum americanum</i>	—	—	—	—	—	—
Northern prickly ash	—	E	—	—	—	—

Letter designations: A = Overstory, fall, 1976

B = Understory, fall, 1976

C = Ground cover, fall, 1976

D = Ground cover, spring, 1977

E = Observation only

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***Lycopodium Flabelliforme* in Central Indiana and Illinois**

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Introduction

Lycopodium flabelliforme (Fern.) Blanch., the common Ground Pine, is a wide ranging species found throughout northeastern United States and adjacent Canada, and has been reported from as far north as Newfoundland and Quebec, from as far south as Georgia and Louisiana, and from as far west as Iowa (7). Though it has an extensive range, it was considered extremely rare in the midwest, and until 25 years ago was known from only one county in Illinois (9), and seven counties in Indiana (3).

This taxon was first reported in Illinois from a sandstone ledge above Lusk Creek in Pope County (10), and until recently this was considered the only known site for this species in Illinois (4, 9). Since that time it has been reported from scattered localities throughout the state. These include three sites in Cook and DuPage Counties (5), one native station in Ogle County (14), an adventive colony in Crawford County (16), and associated with *Liparis liliifolia* in northern Illinois (18). In most of these reports the Ground Pine was considered an adventive, being associated with conifer plantations. Recently, this species was considered to be native to sandstone ledges in Pope and Ogle Counties, and adventive under conifers in Cook, DuPage, and several southern Illinois counties (15). By 1940 this taxon was known from seven counties in Indiana, being extremely local and restricted to moist, rocky slopes (3). Recently it was listed as an adventive in Spencer County (13), Brown County (11), and Vigo County (8).

In the last five years the present author and his students have found 57 colonies of Ground Pine in east-central Illinois and adjacent Indiana. Some of these populations are in conifer plantations, but most are associated with the early tree stage of old-field succession. On some of these sites it is becoming a very aggressive species with the population covering an area of 20 m in diameter. During the present study 25 of these sites were studied to try and determine some of the factors responsible for the recent population explosion of this taxon, and to analyze some of its habitat requirements.

Materials and Methods

Each of the 25 populations of *Lycopodium flabelliforme* were visited three times during the growing season of 1976. On the first visit to each site a quadrat 25 m on each side was marked off with the Ground Pine colony centered in the quadrat. In each of these plots the number, size to the nearest 1/10 of an inch, and species of the woody plants above 1 inch d.b.h. was recorded. From this data the number of trees per quadrat, the basal area per quadrat, the average tree

diameter per quadrat, and the importance value (IV) of each species per quadrat was calculated. As used here the IV is the sum of the relative density and the relative dominance (12). Also, the age of the oldest tree was obtained by taking increment borings from the larger trees in each quadrat.

The vines and shrubs present in each quadrat was recorded during one of the visits to each site. Also, the herbaceous species found growing in the colonies of *Lycopodium flabelliforme* was noted during each visit. From this data the frequency of the taxa commonly growing in association with the Ground Pine was determined. Voucher specimens of all species found are deposited in the Stover Herbarium of Eastern Illinois University (EIU), while the taxonomic nomenclature used follows that of Mohlenbrock (15).

The soil characteristics of each site were obtained from surface soil cores 10 cm deep from within each quadrat. Particle size analyses were made on 50 g aliquots by using the hydrometer method (2), and a soil texture diagram was then used for soil class determination (6). Soil pH was obtained by using a Beckman glass electrode pH meter on a 1:1 soil-water paste (17), and the amount of organic material was determined by the moderate temperature, dry-combustion, resistance furnace method (1).

Results and Discussion

A total of 36 species of herbaceous plants were found growing directly in the colonies of *Lycopodium flabelliforme*, while 16 species of shrubs and vines, and 46 species of trees were observed in the quadrats. The most common of these taxa and their frequencies are listed in Table I. A more complete analysis of the woody overstory vegetation, as well as the soil characteristics of the study plots is included in Table II. In this table the texture, organic content, and pH of the A horizon of the soil is given along with the number of woody stems, the basal area, the average diameter, the age of the oldest trees, and the importance values (IV) of the leading dominants found in each of the quadrats.

Soil Characteristics: The soils associated with the 25 colonies of Ground Pine are fairly similar (Table II). All are loam or silt loam soils in which the silt content varies from 42% to 79% and the clay separate is a very minor component (4-17%). In these soils the amount of organic material varies from 2.1% to 3.6%, and averages 2.6% for all the quadrats. The soil pH is more variable, but always acidic, varying from 3.7 to 6.3 with an average of 5.6. This indicates that *Lycopodium flabelliforme* could become established in most upland old-fields with slightly acid, silt or silt loam soils with a moderate organic content.

Herbaceous Vegetation: The herbaceous plants found in the general area of the Ground Pine populations are the typical taxa associated with the pioneer tree stage of upland old-field succession. The most commonly encountered of these taxa found growing in the Ground Pine colonies are listed in Table I, Part A. In all cases there were very few individuals present, being widely scattered throughout the colony. This is probably due to the dense Ground Pine population which may prevent germination and development of many herbaceous species, but it is possibly due to allelopathy conditions created by *Lycopodium flabelliforme*. It was commonly observed that the herbaceous taxa

TABLE I Frequency of the most common species of plants found in the 25 study plots of *Lycopodium flabelliforme*.

A. Herbaceous plants commonly growing in association with colonies of <i>Lycopodium flabelliforme</i> .			
SPECIES			FREQ.
<i>Potentilla simplex</i> Michx.			80
<i>Panicum lanuginosum</i> Ell.			64
<i>Asplenium platyneuron</i> (L.) Oakes.			52
<i>Achillea millefolium</i> L.			44
<i>Desmodium paniculatum</i> (L.) DC.			44
<i>Galium circaezans</i> Michx.			36
<i>Solidago canadensis</i> L.			36
<i>Pycnanthemum tenuifolium</i> Schrad.			32
<i>Solidago nemoralis</i> Ait.			32
<i>Schizachyrium scoparium</i> (Michx.) Nash.			28
<i>Liparis liliifolia</i> (L.) Rich.			28
<i>Daucus carota</i> L.			24
<i>Poa compressa</i> L.			24
<i>Poa pratensis</i> L.			24
B. Shrubs and vines commonly found in the study plots.			
SPECIES			FREQ.
<i>Toxicodendron radicans</i> (L.) Kuntze.			64
<i>Rubus allegheniensis</i> Porter.			60
<i>Rhus glabra</i> L.			56
<i>Vitis aestivalis</i> Michx.			40
<i>Corylus americana</i> Walt.			36
<i>Rosa multiflora</i> Thunb.			24
<i>Rhus copallina</i> L.			20
C. Trees commonly found in the study plots.			
SPECIES	COMMON NAME	SPECIES SYMBOLS	FREQ.
<i>Prunus serotina</i> Ehrh.	Wild Black Cherry	BC	92
<i>Sassafras albidum</i> (Nutt.) Nees.	Sassafras	Sa	92
<i>Acer rubrum</i> L.	Red Maple	RM	80
<i>Ulmus americana</i> L.	American Elm	AE	80
<i>Fraxinus americana</i> L.	White Ash	WA	76
<i>Quercus velutina</i> Lam.	Black Oak	BO	60
<i>Cornus florida</i> L.	Flowering Dogwood	FD	56
<i>Ulmus rubra</i> Muhl.	Slippery Elm	SE	56
<i>Juniperus virginiana</i> L.	Red Cedar	RC	52
<i>Liriodendron tulipifera</i> L.	Tulip Tree	TT	52
<i>Nyssa sylvatica</i> Marsh.	Sour Gum	SG	44
<i>Diospyros virginiana</i> L.	Persimmon	Pe	40
<i>Quercus imbricaria</i> Michx.	Shingle Oak	SO	40
<i>Acer saccharum</i> Marsh.	Sugar Maple	SM	36
<i>Amelanchier arborea</i> (Michx. f.) Fern.	Shadbush	Sh	36
<i>Quercus rubra</i> L.	Red Oak	RO	36
<i>Betula nigra</i> L.	River Birch	RB	12
<i>Ostrya virginiana</i> (Mill.) K. Koch.	Hop Hornbeam	HH	12
<i>Malus ioensis</i> (Wood) Britt.	Iowa Crab Apple	IC	4

TABLE II Soil and Overstory Conditions at *Lycopodium flabelliforme* Sites in Central Indiana and Illinois.

OVERSTORY (Trees 1" and above d.b.h.)																			
SOIL (A horizon)																			
Site	Location	Texture			Soil Class	% Organic Content	pH	# Ind. Per Site	Basal Area Per Site (sq. ft.)	Av. Diam. Inches	Age Oldest Tree	Species First in IV			Species Second in IV			Species Third in IV	
		% Sand	% Silt	% Clay								Sp. ¹	IV	Sp.	IV	Sp.	IV	Sp.	IV
CRAWFORD CO. ILL.																			
1	Sect. 4 R12W T5N	45	49	6	loam	2.5	5.2	45	2.67	3.0	23	RB	157	WA	31	RM	5		
2	Sect. 3 R12W T5N	32	50	17	loam	2.5	4.8	55	7.07	3.6	22	BO	47	WA	33	RB	30		
3	Sect. 35 R12W T6N	33	57	10	silt loam	2.2	5.7	43	6.52	4.4	32	Sa	137	RM	39	SO	13		
4	Sect. 25 R12W T6N	47	42	11	loam	2.6	4.3	64	2.85	2.6	32	Sa	144	Pe	39	RM	5		
5	Sect. 7 R10W T5N	48	46	6	loam	2.1	5.2	30	2.45	3.5	9	TT	168	SM	28	BC	4		
6	Sect. 4 R12W T5N	49	45	6	loam	2.1	4.6	45	1.48	2.2	14	HH	51	RB	41	Sa	19		
7	Sect. 36 R12W T6N	14	79	7	silt loam	2.6	5.0	27	1.31	2.7	22	Pe	101	Sa	68	RM	17		
8	Sect. 36 R12W T6N	27	65	8	silt loam	3.4	4.4	72	5.88	3.4	32	RM	85	Sa	60	Pe	24		
9	Sect. 1 R12W T5N	27	64	9	silt loam	2.3	3.7	30	7.81	5.8	50	RO	64	BO	40	SO	33		
10	Sect. 12 R12W T5N	27	63	10	silt loam	2.2	3.9	38	4.70	3.3	56	Sa	91	RM	48	SO	35		
RICHLAND CO. ILL.																			
11	Sect. 2 R9E T12N	38	49	13	loam	2.4	4.8	80	5.99	3.2	19	RM	161	Sa	17	Pe	13		
12	Sect. 2 R9E T12N	37	51	12	loam	2.7	4.5	84	4.22	2.5	23	Pe	74	RM	69	Sa	26		
MONTGOMERY CO. IND.																			
13	Sect. 11 R6W T17N	16	75	9	silt loam	2.7	5.9	55	6.60	3.9	20	TT	63	SM	36	RM	36		
14	Sect. 1 R6W T17N	14	78	8	silt loam	3.0	6.1	67	4.87	2.8	22	TT	77	WA	35	Sa	19		
15	Sect. 1 R6W T17N	16	73	11	silt loam	2.2	6.1	71	4.95	2.8	28	Sa	39	SM	38	TT	33		
16	Sect. 10 R6W T17N	17	74	9	silt loam	2.7	5.4	62	2.06	2.1	26	IC	80	FD	60	Sa	14		
17	Sect. 10 R6W T17N	15	71	14	silt loam	2.7	5.6	38	1.70	2.6	25	FD	112	RM	24	Sa	24		
18	Sect. 10 R6W T17N	16	71	13	silt loam	3.7	5.8	21	4.05	4.8	26	TT	56	RM	30	Sa	29		
PARK CO. IND.																			
19	Sect. 3 R8W T16N	23	63	14	silt loam	2.3	5.7	55	5.06	3.2	22	TT	65	RM	63	Sa	22		
20	Sect. 3 R8W T16N	45	45	10	loam	3.6	5.5	29	1.39	2.5	16	RM	84	TT	58	WA	27		
21	Sect. 28 R7W T17N	14	76	10	silt loam	2.4	5.2	76	6.25	3.4	21	RM	114	TT	42	RC	16		

TABLE II Soil and Overstory Conditions at *Lycopodium flabelliforme* Sites in Central Indiana and Illinois. Continued

Site	Location	SOIL (A horizon)										OVERSTORY (Trees 1" and above d.b.h.)												
		Texture										# Ind. Per Site	Basal Area Per Site (sq. ft.)	Av. Diam. Inches	Age Oldest Tree	Species First in IV			Species Second in IV			Species Third in IV		
		% Sand	% Silt	% Clay	Soil Class	% Organic Content	pH	Sp. ¹	IV	FD	Sp.					IV	TT	Sa	WA	SG				
VIGO CO. IND.																								
22	Sect. 19 R9W T13N	20	71	9	silt loam	2.5	6.3	52	8.40	4.3	33	TT	69	BO	36	WA	27							
23	Sect. 18 R7W T13N	22	71	7	silt loam	2.2	5.3	37	1.71	2.6	24	TT	116	FD	33	Sa	26							
24	Sect. 18 R7W T13N	47	47	6	loam	2.2	5.2	61	1.87	2.0	30	FD	104	Sa	87	TT	4							
25	Sect. 18 R7W T13N	49	47	4	loam	2.1	5.3	51	4.23	3.3	27	FD	78	TT	47	SG	38							

¹For species symbols see part C of Table I.

in the colonies were stunted and less well developed than the individuals that were not associated directly in the Ground Pine.

A total of 36 herbaceous species were found growing in the Ground Pine populations. Of these taxa, none were found in all of the colonies. The most commonly encountered species include *Potentilla simplex* which occurred in 80% of the colonies, *Panicum lanuginosum* in 64%, *Asplenium platyneuron* in 52%, and *Achillea millefolium* and *Desmodium paniculatum*, both in 44% of the colonies. Besides the taxa listed in Table I, Part A, some of the species occasionally encountered include *Agrimonia parviflora* Ait., *Botrychium dissectum* Spreng. var. *obliquum* (Muhl.) Clute., *Cassia fasciculata* Michx., *Danthonia spicata* (L.) Beauv., *Eupatorium perfoliatum* L., *Fragaria virginiana* Duchesne., *Geum canadense* Jacq., and *Panicum microcarpon* Muhl.

Woody Vegetation: A total of 16 species of shrubs and vines were found in the quadrats. The most commonly encountered of these taxa and their frequencies are listed in Table I, Part B. Besides these species listed, some of the taxa occasionally encountered include *Celastrus scandens* L., *Hydrangea arborescens* L., *Lonicera japonica* Thunb., *Rhus typhina* L., and *Viburnum prunifolium* L.

Forty-six tree species were also found within the quadrats studied. Of these taxa, only 31 have individuals exceeding 1 inch d.b.h., and the most commonly encountered are listed in Table I, Part C, along with their frequencies. In Table II a more detailed analysis of the overstory is given. For the most part the overstory is that which typically occurs in upland old-fields throughout east-central Illinois and adjacent Indiana. In the quadrats there is an average of 51.5 individuals, the total basal area averages 4.24 square feet, the average diameter rarely exceeds 4 inches d.b.h., while the age of the oldest tree average 26 years (Table II). Also listed in Table II are the taxa that form the dominant overstory (those ranking first, second, or third in IV) in the quadrats. The relative importance of the overstory trees can be determined by considering the number of quadrats on which each species is the leading dominant (highest IV), or ranks second or third. Of the species encountered, *Sassafras albidum*, *Acer rubrum*, and *Liriodendron tulipifera* have the highest IV's in 15 of the quadrats, and rank second or third on most of the other quadrats.

***Lycopodium flabelliforme* Colonies:** The colonies studied vary greatly in size. Two consist of a single individual with a horizontal stem to 5 dm long and a few erect stems. The remaining colonies are much larger, usually circular in outline, and vary from about 1 m to nearly 20 m in diameter. In these colonies the erect stems form a dense mat in which few other taxa grow.

Very little is known concerning the rate of growth of the Ground Pine colonies. However, since the colonies are nearly circular in outline it is likely that they started from a single individual and all subsequent growth is asexual as a result of the creeping, horizontal stems. One colony (site 19) located in the Alle Memorial Woods, Park County, Indiana was first observed during the summer of 1958. At that time the colony was nearly circular in outline and about 1 m in diameter (Dr. Marion Jackson, Indiana State University—personal communication). When this colony was measured during the summer of 1976 it

was still nearly circular in outline, and 15.2 m in diameter. This indicates that the average growth per year was nearly 4 dm. Using this rate of growth, some of the larger colonies examined during the present study are at least 20 years old.

The reason for this invasion of Ground Pine into oldfields in Indiana and Illinois is not known, but is probably the result of an increased planting of pines about 25 years ago. The spores and young plants of *Lycopodium flabelliforme* were probably attached to the roots of the pines, or mixed with the packing material, and became established after planting. Many of the early reports for this species list it as adventive under pines, and many of the sites examined during the present study are in the general area of pine plantations.

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Synopsis of *Heliomeris* (Compositae)

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Introduction

Heliomeris was established by Nuttall (1848) based upon *H. multiflora*. Bentham and Hooker (1873) included *Heliomeris* in *Gymnolomia*, and the species now accepted as members of *Heliomeris* were treated under *Gymnolomia* by Robinson and Greenman (1899). Blake (1918), realizing that *Gymnolomia* was a highly unnatural genus, transferred several species to *Viguiera* as the section *Heliomeris*. Cockerell (1918), primarily on the basis of Blake's work, resurrected the genus *Heliomeris* and transferred all of the species of Blake's section *Heliomeris* to it. He also called attention to the color reaction of the rays of *H. multiflora* in caustic potash which is different from that of *Viguiera*, going on to say that, in spite of Blake's objections, he considered chemical characters as valid as morphological ones in taxonomic work. Until the present, Blake's treatment has been generally accepted. We feel, however, that there is justification for separating *Heliomeris* from *Viguiera*. Species of *Heliomeris* are characterized by having a weakly two-seriate, scarcely graduate involucre with herbaceous, generally lanceolate or linear-lanceolate phyllaries, whereas in *Viguiera* the involucre is two to seven-seriate, more conspicuously graduate, and the phyllaries are ribbed or indurate, at least at the base. Moreover, all species of *Heliomeris* are epappose; a few species of *Viguiera* may also lack a pappus but these are clearly related to other species that have a pappus. It may also be significant that all species of *Heliomeris* thus far counted have a base number of eight or nine chromosomes, whereas in *Viguiera* all species thus far known cytologically have a higher base number. *Heliomeris*, as here interpreted, comprises five species, limited to Mexico and the western United States.

A revision was finished some years ago (Yates 1967) and publication was delayed in the hope that additional field and herbarium study might be undertaken. Although this has proven possible only to a limited extent, it now seems desirable that some of the observations be put on record. For the most part only the name bringing synonyms and those synonyms not properly disposed of by Blake (1918) are included.

Chromosome Numbers

Several chromosome counts of $n = 8$ have been reported for three species of *Heliomeris*, and single counts of $n = 9$ and $n = 16$ have also been reported in *H. multiflora*. Counts for *Viguiera* are based on $x = 16$ or 17 (Federov, 1969; Moore, 1973, 1974) with the exception of *V. tenuis* which is $n = 12$ (Turner and King,

1964). It should be pointed out, however, that chromosome numbers have been reported for only 18 of more than 100 species of *Viguiera*.

H. multiflora. Since the first report of $n = 8$ (Heiser and Smith, 1955), this count has been verified many times. In the present study counts of $n = 8$ were obtained for *H. multiflora* var. *multiflora* from 16 different populations from Arizona, Colorado, New Mexico and Utah. Detailed citations may be found in Yates (1967); voucher herbarium specimens for these counts and the others reported here by us are deposited at Indiana University. Counts of $n = 8$ were also obtained for *H. multiflora* var. *nevadensis* in Coconino Co. (Yates 2094, 2099) and Yavapai Co., (Yates 2076), Ariz., and for *H. multiflora* var. *brevifolia* from Cochise Co., Ariz. (Heiser 63V7). Solbrig *et al.* (1972) report a count of $n = 8$ from California and $n = 16$ from Nevada for *H. multiflora* var. *nevadensis*, and Keil and Pinkava (1976) give counts of both $n = 8$ and 9 for *H. multiflora* from Arizona.

H. longifolia. A count of $n = 8$ was obtained for *H. longifolia* var. *longifolia* from Cochise Co., Ariz. (Heiser 6451), the same as has been reported by others (Heiser, 1963; Powell and Sikes, 1970; Solbrig *et al.*, 1972; Turner and Flyr, 1966; Turner, Powell and King, 1962). Counts of $n = 8$ were obtained for *H. longifolia* var. *annua* from Gila Co. (Yates 2107) and Yavapai Co. (Yates 2108), Arizona, and McKinley Co., New Mexico (Yates 2132). This agrees with the report from Navajo Co., Arizona by Keil and Pinkava (1976).

H. hispida. A count of $n = 8$ was obtained from material collected in Utah Co., Utah (Yates 71-UI2). Keil and Pinkava (1976) have reported this species (as *Viguiera ciliata*) from Sonora as $n = 8$.

Hybridization

Artificial hybridizations were attempted with *H. longifolia* and *H. multiflora* in the greenhouse. The source of the material was as follows: *H. multiflora* var. *multiflora* (V4695), Bernalillo Co., N.M.; var. *nevadensis* (2094), Yavapai Co., Ariz.; var. *brevifolia* (63V7), Cochise Co., Ariz.; *H. longifolia* var. *longifolia* (6451), Cochise Co., Ariz.; var. *annua* (2108), Yavapai Co., Ariz. Both species proved to be self-incompatible so emasculation was unnecessary. Considerable difficulty was experienced in securing germination of the seeds of the annual species and those hybrids with them as the maternal parent. The results are given in Table I.

Intervarietal hybrids of *H. multiflora* gave rather high pollen stainabilities, and, as would be expected, meiosis was normal. Hybrids of *H. multiflora* with the two varieties of *H. longifolia* showed highly reduced pollen stainabilities. Both sets of hybrids were largely morphologically intermediate between their parents and proved to be perennials. Meiosis was examined in both combinations. Eight bivalents were observed at diakinesis but abnormalities became evident at anaphase I. Most cells showed one, or occasionally more, chromatin bridges and one or more lagging chromosomes and possibly acentric fragments. It is likely that the abnormal disjunction is responsible for the sterility, although cryptic structural hybridity cannot be ruled out. The possible presence of acentric fragments along with the chromatin bridges suggests

heterozygosity for a pericentric inversion. Sister crosses of these interspecific hybrids gave no seed set, nor did backcrosses of the hybrids to the annual parents. However, in backcrosses of *multiflora* x *longifolia* to *multiflora* four seeds were produced in six heads. All seeds germinated; two of the seedlings died from damping off, but the other two survived to produce vigorous plants with pollen stainabilities of 77 and 80%. Eight filled achenes were secured in the backcross of *multiflora* x *annua* to *H. multiflora*, but the seeds failed to germinate.

TABLE I Results of hybridizations of *Heliomeris*

Combination	Number of hybrids	Pollen stainability	
		range	average
mult nev x mult mult	4	88-96	93
mult brev x mult mult	6	86-94	91
mult nev x mult brev	5	78-89	84
long ann x mult mult	6	7-33	18
long ann x mult brev	2	17,20	19
long ann x mult nev	7	4-14	8
long long x mult mult	8	12-34	25
long long x mult brev	7	7-33	23
long long x mult nev	2	37,43	40
long long x long annua	11	29-56	44

The artificial hybrids between the two varieties of *H. longifolia* also showed reduced pollen stainabilities (Table I), but the hybrids gave good seed set in backcrosses to both parents. Meiosis was similar to that described for the interspecific hybrids involving *H. multiflora* except that fewer cells showed bridges and lagging chromosomes.

Hybridization attempts were also made of the two species of *Heliomeris* with several species of *Helianthus* (*H. agrestis*, *H. debilis*, *H. niveus*, *H. Porteri*) and *Viguiera* (*V. adenophylla*, *V. cordifolia*, *V. dentata*, and *V. stenoloba*). No seeds resulted from any of the crosses.

Key to Species of *Heliomeris*

- a. Perennials with adventitious buds arising from woody caudex 1. *H. multiflora*
- a. Annuals usually lacking adventitious buds b.
- b. Leaves ciliate-margined on basal three fourths or throughout 3. *H. hispida*
- b. Leaves not ciliate-margined or only on basal one fourth c.
- c. Larger leaves crenate-dentate, oblong-ovate or oblong-lanceolate; known only from Veracruz, Mexico 5. *H. obscura*
- c. Larger leaves entire or serrulate, rhombic-ovate to linear-lanceolate d.

- d. Leaves linear-lanceolate to lanceolate; stems usually branched above and polycephalic; United States and Mexico 2. *H. longifolia*
- d. Lower leaves rhombic-ovate; stems branched at or near base giving rise to monocephalic branches; known only from Utah 4. *H. soliceps*
- 1. *Heliomeris multiflora* Nutt., Jour. Acad. Nat. Sci. Phil., ser. 2, 1:171. 1848. (LECTOTYPE: "Rocky Mountains," *Gambel*, GH!)

Typification of *H. multiflora* raises some questions. In the original description Nuttall cited two collections: Upper California, *Gambel* and Rocky Mountains, *Gordon*. A problem arises in that these two specimens may represent what are now considered two distinct varieties: plants from California and most parts of Nevada are characterized by having narrower leaves than those from the Rocky Mountains. Nuttall in his description states that the leaves are narrow and this could well apply to either variety. We have been unable to locate either a *Gambel* collection from California or a *Gordon* specimen in either BM or PH. Robinson and Greenman (1899) cite both collections in their revision. They state that they had examined specimens from GH and US, but neither specimen has been found at either of these herbaria. Blake (1918) indicated *Gambell* (sic) GH as the type collection. There are two sheets at GH, one of which is labeled "Gambel, Rio St. Juan, R. Mts," the other "Gambell, R. Mts." The latter sheet bears Blake's annotation label and can serve as the type in the absence of the specimens cited by Nuttall.

The perennation of *H. multiflora* is the most reliable character for distinguishing this species. When the basal part of the plant is lacking as with many herbarium specimens, it is often difficult to distinguish this species from *H. longifolia*. *H. longifolia* var. *longifolia*, however, often has longer leaves (more than 8 cm) and a thicker stem (more than 7 mm) than does *H. multiflora*. From *H. longifolia* var. *annua*, *H. multiflora* can usually be distinguished by its larger heads (more than 9 mm in diam.). It seems likely that hybridization must occur between the two species which may contribute to the difficulty of identification of some specimens.

Heliomeris multiflora is the most widespread and variable species in the genus. Four intergrading varieties may be recognized.

Key to Varieties of *H. multiflora*

- a. Larger heads (excluding rays) 1.4 cm or more in diam; leaves ovate-lanceolate; outer phyllaries exceeding head, reflexed 1a. var. *macrocephala*
- a. Larger heads usually less than 1.4 cm in diam.; leaves various; outer phyllaries seldom exceeding heads or only slightly so, erect to slightly reflexed b.
- b. Leaves ovate to elliptic, mostly alternate, apices obtuse, often with small mucro 1c. var. *brevifolia*
- b. Leaves lanceolate to linear, mostly opposite, apices acute, lacking mucro c.

- c. Leaves ovate-lanceolate to linear-lanceolate, margins not revolute 1a. var. *multiflora*
- c. Leaves linear to linear-lanceolate, margins revolute 1b. var. *nevadensis*
- 1a. *Heliomeris multiflora* var. *multiflora*.
Heliomeris multiflora var. *latifolia* Porter, U.S. Dept. Int., Misc. Publ. 4:72. 1874. (HOLOTYPE: Colorado, foothills near Colorado Springs, Porter, location unknown).
Heliomeris multiflora var. *genuina* Blake, Contrib. Gray Herb. 54:109. 1918.
 Southwestern Montana, eastern Idaho, western Wyoming, Colorado, Utah, eastern Nevada, western Arizona, New Mexico, Texas (Culberson Co.) and Mexico (states of Chihuahua, Durango, Jalisco, San Luis Potosi, Sonora, and Zacatecas). 1500-3350 m.
- 1b. *Heliomeris multiflora* var. *nevadensis* (A. Nels.) Yates, comb. nov.
Gymnolomia nevadensis A. Nels., Bot. Gaz. 37:271. 1904. (HOLOTYPE: Nevada, Meadow Valley Wash., L.N. Goodding 968, RM, dupls. at GH! US!)
Viguiera multiflora var. *nevadensis*, Blake, Contrib. Gray Herb. 54:110. 1918.
Heliomeris nevadensis Ckll., Torreyia 18:183. 1918.
 New Mexico, Arizona, southwestern Utah, Nevada, and adjacent California, 1200-2400 m. This variety is sympatric with the previous one except in the western part of its range.
- 1c. *Heliomeris multiflora* var. *brevifolia* (Greene) Yates, comb. et stat. nov.
Gymnolomia brevifolia Greene ex Woot. & Standl., Contrib. U.S.N.H. 16:190. 1913. (HOLOTYPE: New Mexico, Socorro Co., Mogollon Mts., West Fork of the Gila River, Metcalfe 511, ND, dupls. at GH! US!).
Viguiera ovalis Blake, Contrib. Gray Herb. 54:110. 1918 (based on *G. brevifolia* Greene).
Heliomeris brevifolia (Greene) Ckll., Torreyia 18:183. 1918.
 Utah (Beaver Co.), Arizona (Cochise Co.) and New Mexico (Socorro Co.), 2400-3050 m.
- The morphological study and the results of the artificial hybridizations indicate that this taxon is better treated as a variety than as a species. The plants are generally found growing in shaded areas. It was originally thought that the broad leaves might simply represent an ecological modification of phenotype, but plants of the accession grown in the full sun at Bloomington, Indiana, have maintained the distinctive leaf type.
- 1d. *Heliomeris multiflora* var. *macrocephala* Heiser, var. nov. Ab aliis varietatibus capitulis majoribus (1.4-2.0 cm diam) differt.
 Mexico: Chihuahua, "conifer forest on summit of Sierra Mohinora, 10,000-13,000 ft.," Correll and Gentry 23170 (HOLOTYPE, TEX). Same locality, Correll and Gentry 23145 (TEX). La Rocha, along tributary of Rio del Soldado, Sierra Mohinora, Correll and Gentry 23134 (TEX).

The most distinctive feature of this variety is its larger heads. Although the other phyllaries may somewhat exceed the disk and become reflexed in the other varieties, this tendency is most pronounced in var. *macrocephala*. The leaves are also broader than is usual in var. *multiflora* and var. *nevadensis*. *Correll and Gentry 23230* (TEX) from three miles south of La Rócha is somewhat transitional between var. *macrocephala* and var. *multiflora*.

2. *Heliomeris longifolia* (Rob. & Greenm.) Ckll., *Torreyia* 18:183. 1918.
Gymnolomia longifolia Rob. & Greenm., *Proc., Bost. Soc. Nat. Hist.*
 29:92. 1899. (LECTOTYPE: "Western Texas," *Wright 328*, GH!
 [Blake, 1918])

Although Blake (1918) recognized *H. longifolia* and *H. annua* as distinct species and in spite of the reduced fertility in one cross between the two, it seems desirable to recognize these as varieties because of their extensive intergradation in Texas and parts of Mexico.

Key to Varieties of *H. longifolia*

- Some leaves usually 8 cm or more long; branch-
 ing usually confined to upper half of plant;
 heads 9-14 mm in diam.; stem base usually
 more than 7 mm thick 2a. var. *longifolia*
 Leaves usually less than 8 cm long; plants much
 branched; heads 6-9 mm in diam.; stems
 slender 2b. var. *annua*

- 2a. *Heliomeris longifolia* var. *longifolia*
 Western Texas (Brewster, Jeff Davis and Presidio Counties), New Mexico
 (Hidalgo Co.), Arizona (Cochise Co.), and Mexico (states of Chiapas,
 Chihuahua, Durango, Michoacan, San Luis Potosi and Sonora), 600-
 2700 m
- 2b. *Heliomeris longifolia* var. *annua* (M. E. Jones) Yates, comb. nov.
Gymnolomia multiflora var. *annua* M. E. Jones, *Proc. Calif. Acad. Sci.*
 ser. 2, 5:698. 1895. (NEOTYPE: New Mexico, near Defiance, C. C.
Marsh 229, US!)
Heliomeris annua (M. E. Jones) Ckll., *Torreyia* 18:183. 1918.
 Western Texas (Jeff Davis, El Paso and Presidio Counties), New Mexico
 (Dona Ana, Grant, Lincoln, McKinley, Sierra, Taos and Union Counties),
 Utah (Kane and Washington Counties), Arizona (Apache, Cochise,
 Coconino, Gila, Graham, Mohave, Pima, Santa Cruz and Yavapai
 Counties), Mexico (states of Coahuila, Chihuahua, Durango and
 Sonora). 600-2700 m

The original author did not designate a type for this variety. From his account, however, it is apparent that the description of the new variety came about as a result of his visit to the United States National Herbarium. Therefore, we have designated a neotype from among the specimens that he most likely examined.

3. *Heliomeris hispida* (A. Gray) Ckll., *Torreyia* 18:183. 1918.
Heliomeris multiflora var. *hispida* A. Gray, *Pl. Wright* 2:87. 1853.

(HOLOTYPE: Mexico, Sonora, near Santa Cruz and San Bernardino, Wright 1222 GH!)

Gymnolomia hispida (A. Gray) Rob. & Greenm., Proc. Bost. Soc. Nat. Hist. 29:93. 1899.

Gymnolomia hispida var. *ciliata* Rob. & Greenm., Proc. Bost. Soc. Nat. Hist. 29:93. 1899. (LECTOTYPE: Beaver, Utah, Palmer 245 GH! [Blake, 1918])

Viguiera ciliata var. *ciliata* (Rob. & Greenm.) Blake, Contrib. Gray Herb. 54:114. 1918.

Viguiera ciliata var. *hispida* (A. Gray) Blake, Contrib. Gray Herb. 54:114. 1918.

Heliomeris hispida var. *ciliata* (Rob. & Greenm.) Ckll., Torreya 18:183. 1918.

Utah (Millard, Salt Lake, Sanpete and Utah Counties), New Mexico (McKinley and Santa Fe Counties), California (Los Angeles Co.) and Mexico (state of Sonora). 600-2100 m.

This species usually occurs in low damp pastures or meadows, sometimes bordering salt marshes, a habitat quite different from that of the other species. The distinctive pubescence readily sets this species apart from the others.

Two varieties have generally been recognized but there seems little justification to continue to do so, for they appear to differ only in degree of pubescence. The type collection comprises both the very densely pubescent form (var. *hispida*) and the less pubescent form (var. *ciliata*), as was noted by Gray in his original description. The former seems to be rare, for in addition to the type specimen the only other specimen approaching it is *Hasse s. n.* (US) from Santa Monica, California. This species is reported from Arizona (Kearney and Peebles, 1942) on the basis of *Thornber 97* (ARIZ, US) which we would refer to *H. longifolia*. The only recent collection of *H. hispida* that we have seen comes from Utah and possibly it is extinct in some of the other areas where it was known earlier.

4. *Heliomeris soliceps* (Barneby) Yates, comb. nov.

Viguiera soliceps Barneby, Leaf. West Bot. 10:316. 1966. (HOLOTYPE: Utah, Kane Co., Cottonwood Canyon, 1370 m., 41 miles southeast of Cannonville, Barneby 14435 NY!)

This species is known only from Utah. In addition to the type collection we have seen the following from near the type locality: *Reveal et al. 833*, NY; *Welsh and Welsh 12814*, *Welsh 11056*, *Atwood and Allen 02759*, *Atwood and Trotter 5073*, *Atwood 02759*, BRY. As pointed out in the original description the heads of this species are very similar to those of *H. longifolia* var. *annua* but it is very distinct in its vegetative characters from all other species. This species flowers in May and June, whereas all the other species of the United States usually flower much later.

5. *Heliomeris obscura* (Blake) Ckll. Torreya 18:183. 1981.

Gymnolomia obscura Blake, Proc. Amer. Acad. 51:515. 1916. (HOLOTYPE: Mexico, Veracruz, Maltrata, Kerber 211 BM, dupl. at US!)

Viguiera obscura (Blake) Blake, Contrib. Gray Herb. 54:115-116. 1918.

This very distinctive species is known only from the type collection. It does not appear to be particularly closely related to any other species of *Heliomeris*.

Excluded Species

1. *Heliomeris tenuifolia* A. Gray = *Viguiera stenoloba* Blake (fide Blake, 1918).
2. *Heliomeris simplex* Durand, Journ. Acad. Nat. Sci. Phil. Ser. 2, 3:91. 1855.

We have been unable to locate the type collection (California, *Pratten*) but the "disk corollas dark purple" given in the description excludes it from *Heliomeris*, all species of which have yellow disk corollas. Following the description, Durand states: "I have the same plant from the head of Rio Laguno, collected by Dr. Heerman...called by the Mexicans, 'Ojo de Gallo'." A specimen at PH, labeled "head of Rio Laguno [Laguna?], ojo d'gallio, Sept. 21, 1851" without collector being indicated, may possible represent the specimen of which he wrote and it most likely comes from the Sitgreaves Expedition. Durand states that his species lacks a pappus, but this specimen definitely possesses a pappus. This specimen is probably an isotype of *Helianthus praetermissus*, E. E. Wats, which is known only from the type collection.

3. *Heliomeris Porteri* (A. Gray) Ckll., *Torreyia* 18:183. 1918 = *Helianthus Porteri* (A. Gray) Heiser, comb. nov.

This southeastern species was originally described as *Rudbeckia? Porteri* (HOLOTYPE: Stone Mountain, Georgia, *T. C. Porter*, GH!) by Gray (1849), who later placed it in *Gymnolomia* where it remained until Blake (1918) transferred it to *Viguiera*. In general aspect it is rather similar to the species of *Heliomeris*, but the only compelling reason for placing it in the latter genus is the epappose achenes. It is geographically isolated from all species of *Heliomeris*. This alone would not be reason for removing it from the genus. *H. Porteri* has a haploid chromosome number of 17, the same as found in many species of *Helianthus*, and unknown for *Heliomeris*.

Once *Heliomeris* is removed from *Viguiera* there is no justification for retaining this species in *Viguiera*. Moreover, there are no species of *Viguiera* in the southeastern United States. Crosses of *H. Porteri* have been attempted with a few species of *Viguiera* (Heiser, 1963; Yates, unpub.). A single hybrid was secured with *V. adenophylla*, but it was completely sterile and showed 34 univalents at diakinesis.

As this species does not seem at home in either *Heliomeris* or *Viguiera*, another genus must be found for it. The possibilities are to make it a monotypic genus or to transfer it to *Helianthus*. Either course of action might be justified, but we have decided on the latter, for it seems clear that its nearest relatives are to be found in *Helianthus*. Morphologically, it is presently excluded from *Helianthus* solely by the lack of a pappus, so once placed in this genus the generic description will have to be emended. Since the pappus of *Helianthus* is readily deciduous and serves no known function, its loss could hardly be considered a major evolutionary change. *Helianthus* is well represented in the southeastern United States, and it is there that another annual, *H. agrestis*, is found which is

certainly as distantly related to any other species of *Helianthus* as is *H. Porteri*. Moreover, artificial hybrids have been secured of *H. Porteri* with four species of *Helianthus*, and hybrids with *H. niveus* showed an average of 19% pollen stainability and up to ten pairs of chromosomes at metaphase I (Heiser, 1963). The evidence from the hybrids presently available would support its inclusion in *Helianthus*.

4. *Viguiera Shrevei* Steyermark, Field Museum Nat. Hist. **17**:421. 1938 (May 27) (HOLOTYPE: Mexico, Chihuahua, *Shreve 8035*, FM!) = *Verbesina longifolia* A. Gray, Proc. Amer. Acad. **19**:12. 1883 and so annotated by Steyermark on Oct. 29, 1938. In his description, however, Steyermark claimed that his new species apparently is closely related to *H. multiflora*.

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SCIENCE EDUCATION

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Abstracts

How to Produce Your Own Learning Package. STANLEY S. SHIMER, Indiana State University, Terre Haute, Indiana 47809._____Developing learning packages are not as difficult as it may seem. This presentation will demonstrate the steps and techniques for making a 35 mm slide and sound tape program. The author will explain at least three ways the learning package can be utilized in the classroom.

Simple Slide Rule to be Used by Brain Damaged and Other Handicapped Persons. JAMES MITCHELL SMITH, Science Education Section, Indiana Academy of Science._____Only numbers 1 through 10 are used on an expanded slide rule, the spacings of which have been increased four times.

The bulky nature of the rule and the length of the rule give a sense of physical action in working the rule. The photo used with this presentation shows a multiplication of 2 time 3 or a division of 6 by 3. Young persons of very limited ability quickly learn to use the slide rule and enjoy teaching their friends to use the slide rule.

Because only whole numbers are used, this is an intermediate teaching device, attempts to multiply 4 time 6 lead to a situation in which the teacher must step in to help.

The rule can also be used with cards for numbers 1 through 10 each card (about the size of a playing card) has the information, (use the number 2 as an example) *TWO* II 2 · Students place each card with the suitable number on the board.

This rule, together with other teaching material developed by this writer, was used by severely handicapped young persons, several Downs Syndrome youngsters, at New Castle, Indiana over a period of several months.

The assignment of this writer—teaching adult classes in agriculture in another part of the building—was not with the handicapped persons. Any teaching of the young persons was done with the regular teacher present.

It is the thought of this writer that the slide rule can be made commercially and he seeks a sponsor for such a program.

Advertisements and Scientific Analysis. LAWRENCE B. STEWART, Biology Department, Saint Mary's College, Notre Dame, Indiana 46556._____A recent trend can also apply to the teaching of important basic concepts in any science course. There is always room for improving the skills of observing, inferring, and critically or scientifically analyzing information. A good source of

information is advertisements, presented to us in the form of television and radio commercials, or magazine and newspaper ads.

One can teach students to sharpen their powers of observing and critically analyzing through studying the visual and audio messages of ads. Advertising agencies employ subtle techniques to attract the attention and influence the consumer to purchase a product. One can identify the common techniques used by advertising agencies, but more appropriately for a science class, one can study the scientific claims presented in the ads. Advertisers must be able to support a scientific claim with experimental data as the Federal Trade Commission's "Truth in Advertising Law" states.

Studies can be made of scientific claims in ads and tested for verification in the laboratory. Grammatical analyses can also be made of the use of words or phrases in the advertisement for their impact.

The major purpose of this enterprise is to encourage students to question and pay close attention to the way in which advertisements can influence the buying public. This scientific analysis can also help to discourage gullibility on the part of the consumer.

Galvanic Skin Response as a Measure of Emotional Involvement in Biology Instruction. BEVERLY L. KETCHAM and JERRY J. NISBET, Department of Biology, Ball State University, Muncie, Indiana 47306. _____ Evaluation should be comprehensive enough to include all outcomes of learning, not merely those in which learning is most easily assessed. Measurement of emotional responses offers a logical, though indirect, approach to the evaluation of instructional strategies which are intended to facilitate learning in the affective domain.

The function of affective learning situations is to provide a stimulating learning environment in which the student may become emotionally involved. Emotional responses are associated with learning in the affective domain. Internal physiological responses are effective indicators of emotion. These physiological reactions are associated with unconscious responses and can be monitored continuously, whereas verbal and written reports are obtainable only at discrete points within or before and after the learning experience. One method of measuring physiological responses is by monitoring the galvanic skin response (GSR).

Significant, measurable changes occur in the electrical properties of the skin that are closely associated with visceral and neural activities that occur during emotion.

In measuring galvanic skin reactions, electrodes may be attached by velcro strips to the plantar surface of the first and third finger of the non-preferred hand. Resistance per unit area of the electrode plate surface is calculated in ohms/mm² and converted into conductance (mhos/mm²). Increase in conductance indicates a greater emotional involvement, whereas a decrease in conductance indicates a calm, relaxed condition.

We have used GSR to measure emotional responses of students who participated in electronic response (ER) instruction in a biology course at Ball State University. Each of nine ER programs produced a gradual rise in average conductance. Certain combinations of music, narration and 35mm slides

produced higher levels of conductance than other combinations. Answer slides, projected following projection of question slides, produced the highest average conductance recorded in the study.

Learning strategies which produce increased emotional arousal are theoretically the most effective for facilitating learning, increasing remembrance and enhancing retention. GSR measurements have the potential for providing guidance to educators interested in enhancing the impact of their instructional programs.

Piaget, the Learning Cycle, and the Laboratory. JOHN A. RICKETTS, Chemistry Department, DePauw University, Greencastle, Indiana 46135.——Within the Piagetian model intellectual development proceeds through four levels: sensory-motor, pre-operational, concrete operational, and formal operational. Transition from one level to another occurs via the process of dis-equilibration and accommodation. Since the first two levels are normally completed by the age of 7 or 8, it is the last two of the intellectual levels that are of concern. A concrete operational student can perform intellectual operations on objects such as classification and spatial and temporal ordering and can perform the operations of elementary mathematics; he can use the proper algorithm to solve a problem with no idea of why he uses the particular recipe. In addition to being able to operate on objects a formal operational student can reason abstractly using hypotheses and can construct new operations involving propositional logic.

One teaching strategy that facilitates the transition from concrete to formal operational is the "learning cycle." The cycle has three phases: *Exploration* in which the students learn through their own actions and reactions in a new situation, *Invention* in which the concepts recognized as part of *Exploration* are used, and *Application* in which the range of applicability of the *Invention* is extended. In the case of science instruction the "learning cycle" can be employed in the laboratory most effectively. As an example one experiment, "A Chemical Dilemma", is described. Unlike the normal learning cycle which directly involves student-instructor interaction with emphasis on instructor guidance during the *Invention* and *Application* stages, this exercise depends on student-student interaction exclusively.

Science from a Pocketbook. NANCY T. WATSON*, JAMES WATSON, JR., Ball State University, Muncie, Indiana 47306.——Elaborate equipment is not needed to perform simple and interesting science experiment for all levels. The "equipment cabinet" is a lady's pocketbook. A slide presentation will show how Newton's laws, center of gravity, circular motion, sound, electricity and other principles can be demonstrated using "equipment" from a pocketbook.

Human Genetic Education in the Secondary School. S. A. RHINE, and R. M. ANTLEY, Genetics Division, Methodist Hospital, Indianapolis, Indiana and N. S. LANTZ, Marion County Association for Retarded Citizens, Indianapolis, Indiana.——Of 200 normal human conceptions, 80 are lost before the pregnancy is recognized. Of the 120 recognized pregnancies, 20 are lost due to naturally occurring miscarriages and stillbirths. Of the 100 resultant newborn babies, 4(1/25) will have severe birth defects. When these figures are applied to

the >150,000 Juniors and Seniors in Indiana high schools this year, we should expect that they would be involved with >600,000 conceptions; 240,000 early pregnancy losses; 360,000 deliveries; and 12,000 babies with severe birth defects!

We have developed a convocation-slide presentation entitled "The Most Important 9 Months of Your Life" which we hope will reach most of the Juniors and Seniors in Marion County during this school year. Our goal is to promote an understanding of the causes of birth defects, mental retardation, and genetic diseases. Both genetic and acquired etiologies will be elaborated in the program.

Genetic causes are separated into chromosomal, single gene, and multifactorial categories. The basic biologic mechanisms and mistakes which are involved with each category are discussed and examples of affected patients are described. The ever increasing potential for prevention of many of the genetic conditions is pursued by a discussion of genetic counseling and prenatal diagnosis.

The acquired birth defect problems are outlined as environmental insults to the pregnancy which usually affect the fetus via the mother, i.e. alcohol, tobacco, drugs, medications, venereal disease, virus infections, poor nutrition, radiation exposure, environmental pollutants, etc. In addition, perinatal insults such as difficulties during labor and delivery are discussed and the importance of good professional and hospital care during pregnancy and delivery are emphasized.

We hope an evaluation, comments, criticisms, and suggestions by Academy members will help us further develop and improve the program. In addition, we hope to eventually make the program slides and/or a video tape plus a text available to the secondary schools.

Small Computer Application of Pollutant Dispersal Models for Environmental Instruction

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Abstract

Programs were written for a small computer to utilize reported dispersal modeling equations for classroom instruction. The applications were for calculation of downwind concentrations of pollutants, for calculation and plotting of isopleths, constant pollutant concentration contours, and for calculation and labeling of positions on a scaled map of average annual pollutant concentrations. The results obtained for an actual case involving potential sulfur dioxide pollution from a proposed industrial development is reported. The proposed use of these programs for instruction in environmentally oriented courses is discussed.

Introduction

Computer models for atmospheric dispersal of pollutants have come into common use by industries and environmental agencies as a basis for decisions pertaining to industrial developments in relation to environmental protection. There appears a definite need for the incorporation of this material into environmentally related courses at the undergraduate level. This project was undertaken for this purpose and with the understanding that it should be applicable at smaller institutions such as community colleges or branch campuses with limited facilities. Consequently, the programs were developed for a small computer as would be available at almost any institution.

Since the programs were limited to a computer with small core capacity, approximately 4000 hexydecimal words, only point sources of pollutants were considered excluding line and area sources. However, the basic Gaussian diffusion equations were incorporated including plume rise calculation and including provision for wind rose data and the six standard weather stability classes.

Modeling Equations

Employing the standardized symbols for metric units, the basic diffusion equation expresses the concentration $C(\text{g}/\text{m}^3)$ of a given pollutant at a receptor position located by coordinates $X, Y, Z, (m)$, with X the horizontal distance from

¹PROGRAM HPADM-BASIC, 454 statements, transportability not certified; student-run keyboard mode (I/O via keyboard/lineprinter and plotter); execution requires 4K (HP9830A 4K, 16 bit words); plotting output; instruction manual for operation of program with sample run and listing. No flow chart and minimal documentation of program. Copies of the manual (including listing) are available (\$3.00 for copying and postage; checks only—payable to Bursar, Indiana State University Evansville). Will supply only manual with listing. Chemistry Department, Indiana State University-Evansville Campus, Evansville, Indiana 47712.

the stack along the center line of the plume, Y and Z the lateral and vertical distances perpendicular to the center line, respectively. The other variables involved are the emission rate $Q(\text{g/s})$ of the pollutant considered, the effective height of the stack $H(\text{m})$, which includes the plume rise, the wind speed $\mu(\text{m/s})$, and the standard deviations of plume concentration distributions in the horizontal and vertical directions $\sigma_y, \sigma_z(\text{m})$, respectively, which are functions of the downwind distance from the source and of the weather stability class. (1) The basic equation follows as

$$C = \frac{Q}{2\pi\sigma_y\sigma_z\mu} \exp \left[-\frac{1}{2} \left(\frac{y}{\sigma_y} \right)^2 \right] \cdot \left\{ \exp \left[-\frac{1}{2} \left(\frac{z-H}{\sigma_z} \right)^2 \right] + \exp \left[-\frac{1}{2} \left(\frac{z+H}{\sigma_z} \right)^2 \right] \right\}$$

Calculations for the quantities σ_y and σ_z were obtained by fitting low order polynomial approximations to published curve plots. (2). The effective stack height is obtained by adding the plume rise to the stack height. The plume rise is calculated from the Holland equation

$$\Delta H = \frac{V_s d}{\mu} \left[1.5 + 2.68 \times 10^{-3} P d \frac{(T_s - T_A)}{T_s} \right],$$

in which ΔH (m) is plume rise, $V_s(\text{m/s})$ is stack gas velocity, $d(\text{m})$ is the stack diameter, $\mu(\text{m/s})$ is the wind velocity, $p(\text{mb})$ is atmospheric pressure, $T_s(^{\circ}\text{K})$ is stack gas temperature, and $T_A(^{\circ}\text{K})$ is the ambient temperature.

Applications of the Model

The computer employed was a Hewlett Packard model 9830A with BASIC language capability and equipped with line printer and plotter. The programs as developed were stored on a magnetic cassette tape.

The specific programs were developed to apply to a proposed industrial development near a small town in southwestern Indiana. There were already several industries in the locality, and an existing level of atmospheric pollution was apparent. The proposed new development would add significantly to the emission of several pollutants. Three programs were developed to study the impact of the proposed facility to the overall atmospheric pollution in the area.

The first program was developed to calculate the instantaneous concentration of a given pollutant at ground level at a given position for any one of the six weather stability classes and any assumed wind speed for the specified emission parameters of the facility. Provision was made for incrementing the downwind distance so that the maximum concentration and its location could be easily determined from a tabular printout.

The emission data for sulfur dioxide for the proposed plant is summarized in Table I. For a typical wind speed of 12.3 miles per hour, weather stability class D, and ambient temperature of 68° F results were obtained which are presented in Table II. By examination of Table II it is seen that the sulfur dioxide concentration reaches a ground level maximum at approximately 2.5 miles downwind from the stack. This indicates that with a southwest wind, a frequent case for the locality, the maximum pollutant concentration would occur near the center of town.

TABLE I *Emission Data of Proposed Plant*

Emission rate of sulfur dioxide:	34,391 lb/day
Flue gas velocity:	274,090 cu. ft./min.
Stack diameter:	9.5 feet
Stack height:	250 feet
Flue gas temperature:	350.6° F.

TABLE II *Sulfur Dioxide Concentrations from Proposed Industrial Plant at Distance X from Stack*

Distance X (miles)	Concentration of SO ₂ (micrograms/meter ³)
0	0
0.25	0.00
0.5	0.15
0.75	9.89
1	45.9
1.25	91.9
1.5	130.
1.75	155.
2	169.
2.25	175.
2.5	176.
2.75	173.
3	168.
3.25	162.
3.5	155.
3.75	148.
4	142.
4.25	135.
4.5	129.
4.75	123.
5	117.

A second program was developed to calculate and plot isopleths which are contours of constant pollutant concentration. The isopleths only occur over a limited range of concentrations. An existing isopleth has the form of a curve symmetric about the center line of the plume. It originates at the point on the center line where the specified concentration first reaches ground level. If carried far enough downwind the isopleth eventually terminates on the center line forming a closed loop-shaped curve. Higher concentrations occur in the region within the isopleth, so that if a family of isopleths for different concentrations are plotted together they appear with successively higher concentrations approaching the center line more closely.

The same input parameters used with the previous program were applied to the isopleth program. The plotting equipment permitted adjustment of scale so that the isopleths could be plotted to the scale of an available map of the area for direct superposition. (Fig. 1) shows the results obtained for a southwest wind of 12.3 miles per hour and weather stability class D. The five isopleths plotted are for concentrations of 160, 140, 100, 60, and 20 ($\mu\text{g}/\text{m}^3$) and are superposed to

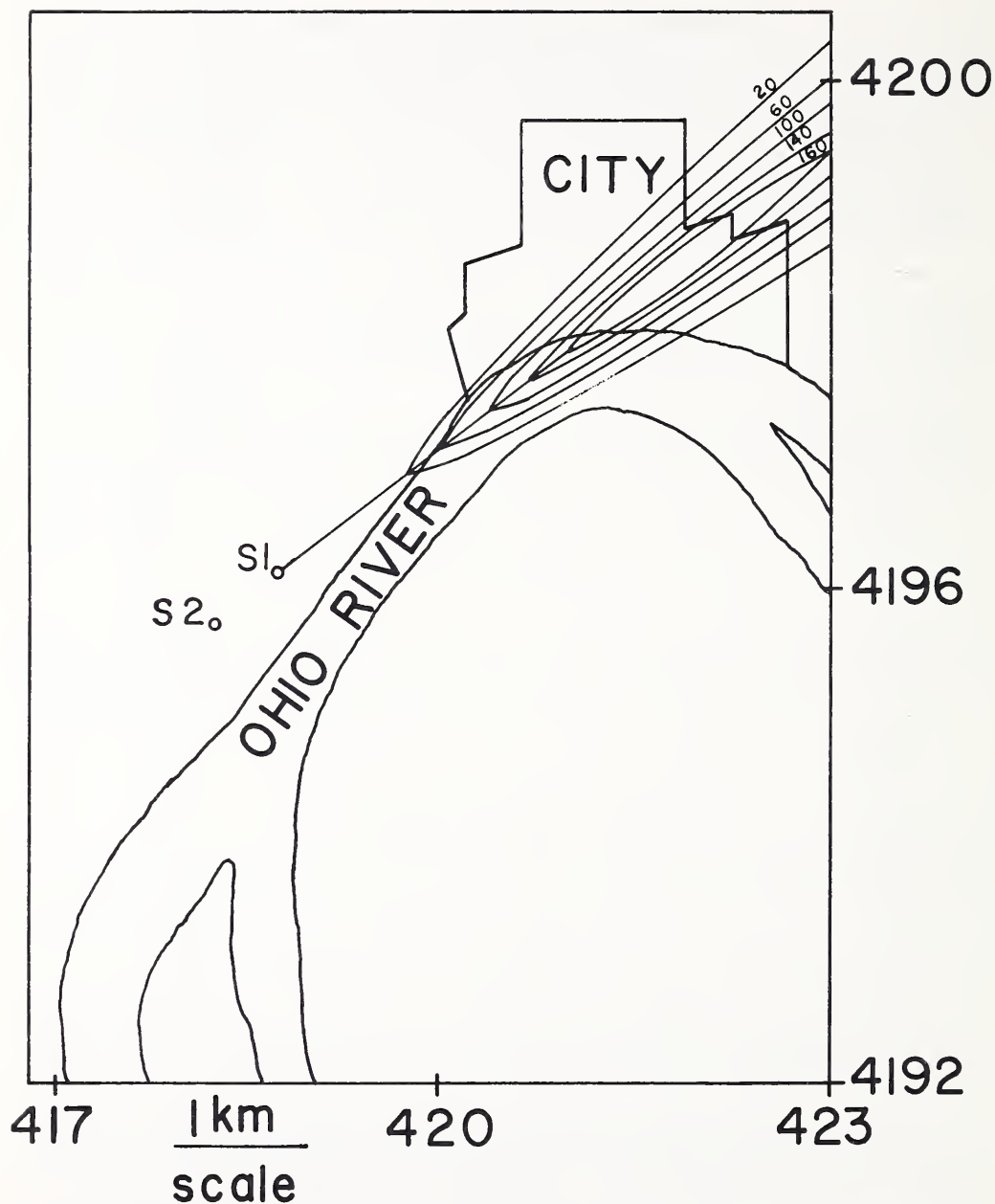


FIGURE 1. Plot of Isopleths of Sulfur Dioxide with concentrations labeled in Micrograms/ m^2 and Axes labeled in Universal Transverse Mercator Coordinates.

scale on an area map of the locality. The maximum concentration is seen to occur in a densely populated section of the town.

Of course, it should be kept in mind that the concentrations involved are strictly instantaneous values. If extended periods are considered lower average values will occur because of variations in the wind direction.

A third program was developed to calculate the average annual concentration of a given pollutant at a given position for the average weather stability class D assumed throughout. The program incorporated averaging over frequencies of wind directions and speeds supplied by the U.S. weather department compiled from a 5-year record for the region. Provision was made

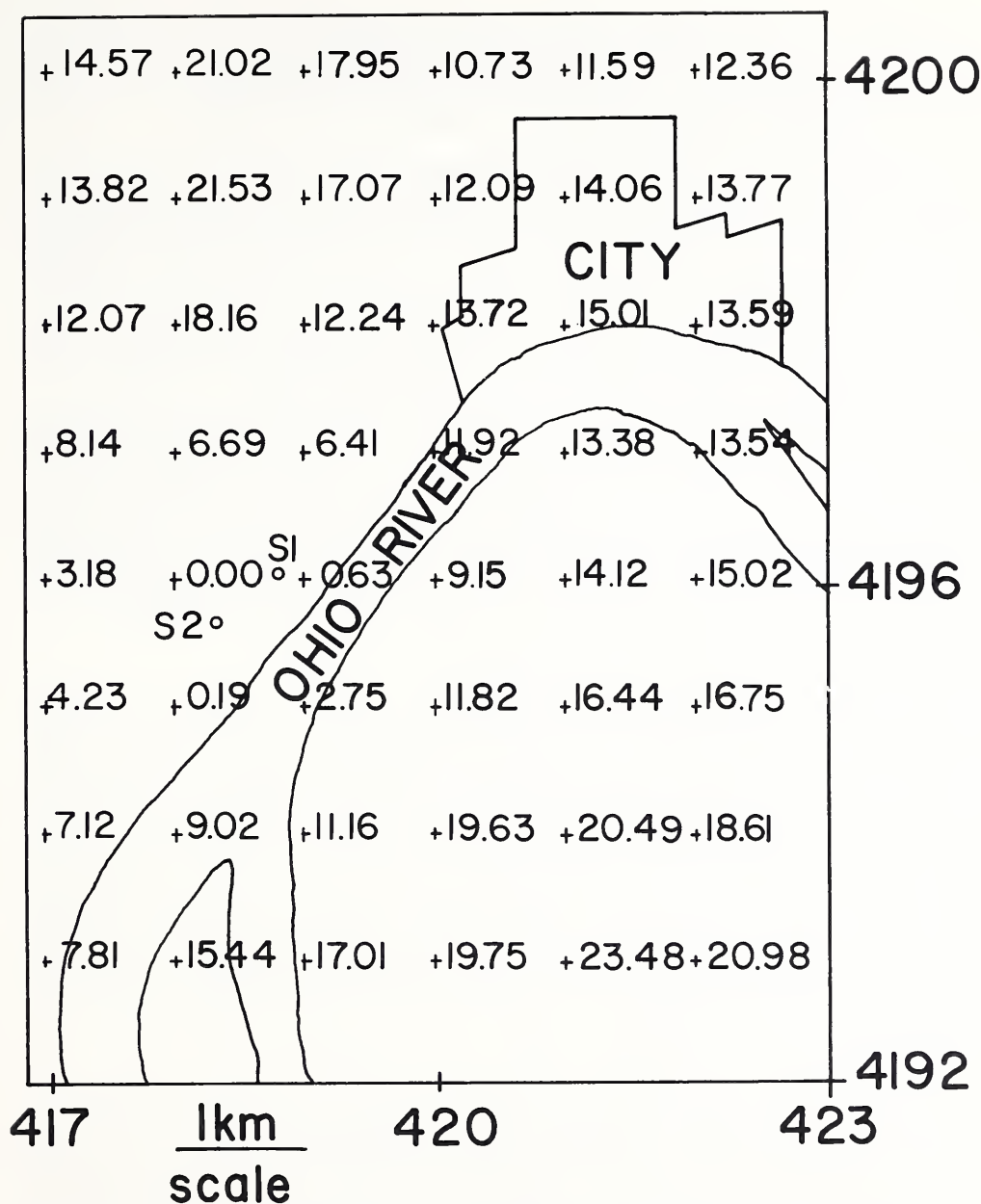


FIGURE 2. Average Annual Concentrations of Sulfur Dioxide in Micrograms/m² with Axes labeled in Universal Transverse Mercator Coordinates.

for accumulating results from a multiplicity of sources. Emission data for the proposed new source and also for the principal existing source maintained by the same company were entered so that the combined effect of both sources could be calculated. Source and receptor positions were expressed in universal transverse Mercator coordinates. Further provision was made for incrementing the receptor position to facilitate an effective scan of the area involved. The program was extended with the capability of labeling concentrations at plotted positions on a scaled map.

A typical result obtained from this program is presented in Fig. 2. The results obtained in this form are very conveniently expressed to facilitate

locating the positions where state and federal ambient air standards would be most likely violated. In this particular case the combined emissions of the two plants are calculated to account for more than 25% of the ambient air standard for sulfur dioxide at this locality.

All results for the case studied indicate that the choices of locations of these plants were poor because frequently occurring winds from the southwest produce high concentrations of pollution in the neighboring metropolitan section. The choices of emission parameters such as the stack heights and flue gas velocities are also such that the downstream concentrations under certain atmospheric conditions can peak at the distances which correspond to the distances from the stacks to population centers in the area.

In all cases it should be kept in mind that all of these calculations are based on a statistical model and represent estimations rather than highly accurate predictions. In some cases results were compared with experimental measurements and also with reported calculations from more sophisticated programs utilizing a larger computer. The results were found to generally agree in order of magnitude but with differences as large as 100% to 200%.

Discussion

Atmospheric pollution is one of the major subject areas taken up in courses in environmental chemistry and other environmentally oriented subjects. With programs of the type presented available to a class, sufficient results can be obtained in a few afternoons from a small low-cost computer to effectively determine the environmental impact on a region from one or more point sources of pollution. The results can be presented in the classroom in the same form as shown for easy comprehension. The computer operations required can be conveniently assigned as student projects to members of the class. The overall effect should be the availability of a large amount of important information at a very low cost in time and effort.

These programs can also be used for other studies than we have reported here. The effects of various weather conditions on the magnitudes and locations of peak concentrations of pollution can be investigated in detail. If a given source is considered for a locality the effect of its location can be studied by assuming different locations and calculating the resulting pollution levels produced in the neighboring metropolitan centers. The effect of emission parameters can be investigated by assuming differing sets of values and calculating the pollution levels that result. Consequently, these programs can also be employed for individual student projects directed toward detailed investigations of more specialized topics.

Literature Cited

1. TURNER, P. BRUCE. 1970. Workbook of Atmospheric Dispersion Estimates. U.S. Department of Health, Education, and Welfare, Cincinnati, Ohio. 5-6.
2. Ibid. 8-9.

Minitransparencies as Teaching Aides

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Science teachers are continually searching for means of presenting information and concepts in science. For several years the author has been making minitransparencies. This paper will outline procedures for making these.

Minitransparencies of this size are more easily stored along with other 2 x 2 photographs. They also can readily be put in a slide projector, with other photograph slides eliminating the use of an overhead projector. Transparencies made by this method can be shown in a fully lighted room.

Prior to production of minitransparencies, an 8½ x 11 ditto carbon is ruled off into twenty squares approximately 2" x 2" or more accurately 4.6 cm x 4.6 cm. Use of the ditto carbon allows practice sheets and final copy sheets (Fig. 1), Photo of layout, to be inexpensively produced. The finished illustration will fit into a Kodak 2 x 2 Ready Mount.

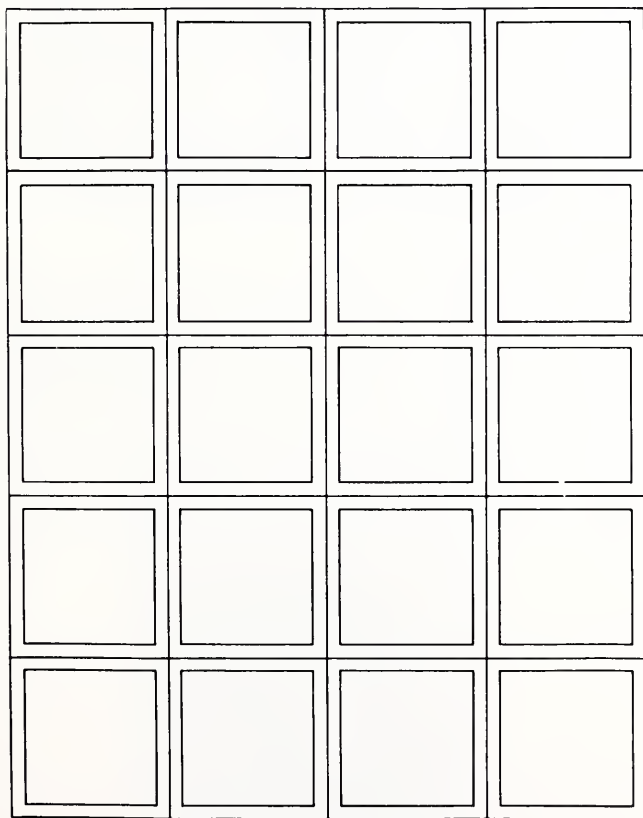


FIGURE 1. *Photo of layout.*

Illustrations may be drawn with pencil, ball point or a Rapid-o-graph drawing pen for greater clarity (Fig. 2) Photo of Sketches.

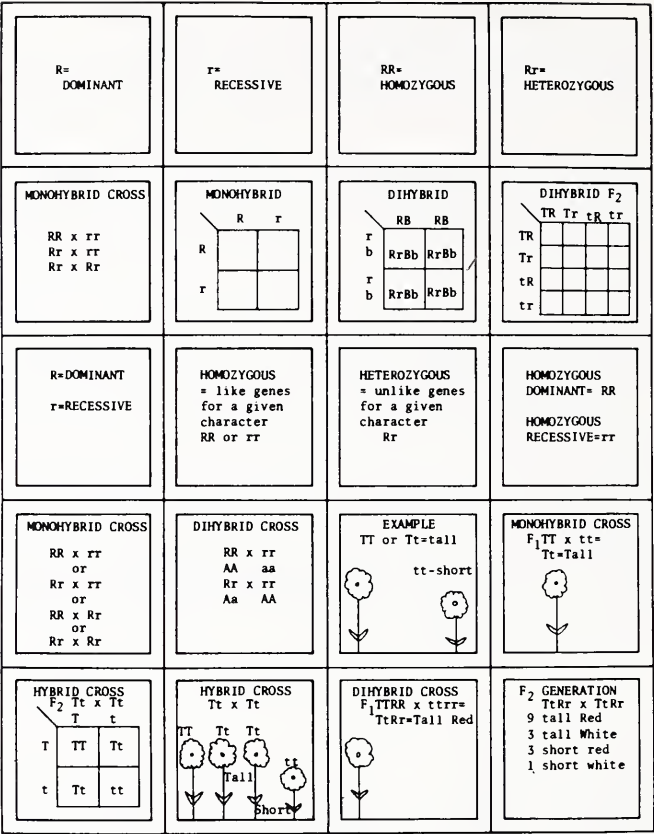


FIGURE 2. Photo of sketches.

After the rough sketches are drawn on the ditto copy sheets they can be copied on transparency film. Using a 3M Dry Photo-Copier Model "107" or other copier. The author uses Dual Spectrum transparency film 3M type 628 (Fig. 3). The intermediate-sheet is removed from the finished transparencies.

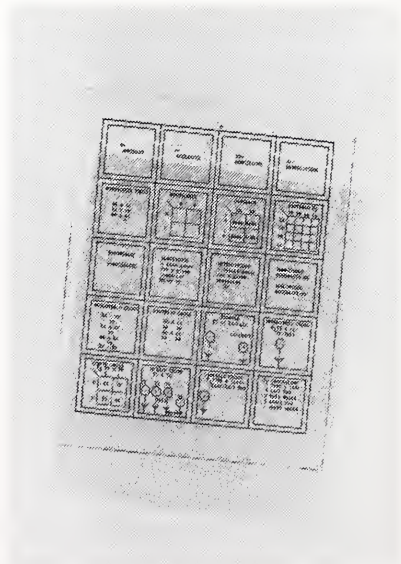


FIGURE 3. The intermediate-sheet removed from the finished transparencies.

The sheet of finished transparencies may now be cut along the outer margin lines of the illustrations, and each minitransparency is ready to be sealed in the ready-mount holder. The minitransparency will slightly overlap the adhesive surface. Close the ready-mount and apply heat with a tacking iron or any common household electric iron. After the transparency is sealed in the mount, rotate it until it is upside down and mark it or number it in the upper right-hand corner. The slide can then be used in any 2 x 2 slide projector (Fig. 4). The final product ready for projection.

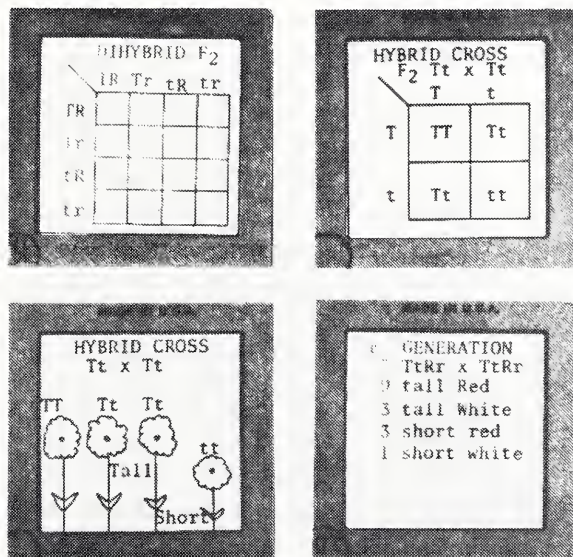


FIGURE 4. The final product ready for projection.

Slide projection by this means is very inexpensive when compared to conventional methods, presently about 6 cents per illustration.

This idea has served the author well in presenting concepts to students in beginning biology and botany courses as well in methods courses for student teachers. Once you have tried this method you will realize the unlimited potential for this technique.

SOIL AND ATMOSPHERIC SCIENCE

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Abstracts

Chemistry and Mineralogy of Some Shale Derived Soils of Southeastern Indiana. J. R. CRUM and D. P. FRANZMEIER, Agronomy Department, Purdue University, West Lafayette, Indiana 47907.——In Clark County, Indiana, it has been recognized by foresters and soil scientists that the soils developed from the gray-green, New Providence shale are very poor forest producers. In this study, soils ranging from poor forest production to high forest production were sampled and characterized in an attempt to determine those properties, chemical and physical, responsible for differences in forest production.

Three soils were studied. The Rarden, developed on the gray-green New Providence shale, is a member of the fine, mixed, mesic Aquic Hapludults and is considered to have a much lower site index (productivity) than its physical properties would suggest. The Trappist (fine, mixed, mesic Typic Hapludult), developed on the black New Albany shale, is considered to be a moderate forest producer. And the Hickory (fine-loamy, mixed, mesic Typic Hapludalf), developed on calcareous Illinoian glacial till, is considered a high forest producer. Each soil was sampled and then characterized by the Purdue Soil Characterization Laboratory.

To supplement the soils study, leaves from dogwood, sugar maple, and white oak trees were collected at each site and analyzed for twelve nutrient and micronutrient concentrations.

The Rarden and Trappist have very low extractable calcium (<1 meq/100 g) and very high extractable acidity concentrations indicative of highly weathered Ultisols. Of the acidifying cations, hydrogen and aluminum, aluminum is by far the dominant cation with contents as high as 14 meq/100 g in the Rarden soil and 10 meq/100 g in the Trappist soil.

We propose that the Rarden's poor forest production is due to extremely low levels of extractable calcium and very high levels of aluminum. the Trappist has better production due to slightly higher levels of calcium and significantly lower levels of aluminum throughout the profile.

Secondary and Micronutrient Test Data of Purdue's Soil Testing Laboratory. RUSSELL K. STIVERS, Department of Agronomy, Purdue University, West Lafayette, Indiana 47907.——The purpose of this study was to determine (1) the number of soil tests for calcium, magnesium, sulfur, boron, manganese, and zinc made, (2) average values for these tests, and (3) percentages of these tests in the low and very low levels as determined by or for the Purdue Plant and Soil

Analysis Laboratory. Four-year (1974-1975 through 1977-1978) annual average numbers of tests made were 816 for calcium and magnesium, 164 for sulfur, 278 for boron, 403 for manganese, and 414 for zinc. For a fifteen month period in 1977-1978 average test values in parts per two million were 3035 for calcium, 549 for magnesium, 23.0 for sulfur, 1.2 for boron, 40.8 for manganese, and 8.0 for zinc. Both strip-mine and ordinary farm samples were included in these averages, but farm samples were less than half of the total for each element. Out of the farm samples tested, the percentages testing low and very low were 2.1 for calcium, 5.4 for magnesium, 59.1 for sulfur, 82.6 for boron, 68.4 for manganese, and 40.0 for zinc. The implications of this study are that soils that are potentially deficient (testing low and very low) in sulfur, boron, manganese, and zinc may be more widespread in Indiana than previously reported.

Algal Availability of Soluble Phosphorous in Drainage Water of the Black Creek Watershed. R. A. DORICH and D. W. NELSON, Purdue University, West Lafayette, Indiana 47907.——The availability of soluble phosphorous to algae was determined in drainage water of the Black Creek Watershed, Allen County, Indiana. Water samples were collected in sterile glass containers from 7 sites within the Black Creek Watershed immediately following rainfall events on March 28 and June 30, 1977. Algal availability was determined in filtered water samples by the Provisional Algal Assay Procedure Bottle Test (PAAP). The PAAP method involves the generation of a reference curve relating the population of *Selanastrum capricornutum* (a single-celled green alga) and P concentration in PAAP nutrient medium after 4 days of incubation. Four days after inoculation of water samples with *S. capricornutum*, cell numbers in the sample are also determined. The quantity of algal available P in the water sample was determined by comparing the number of cells produced in the water sample to the relationship between algal population and P concentrations in the PAAP nutrient medium.

To determine if a micronutrient deficiency was limiting algal growth, water samples inoculated with *S. capricornutum* were spiked with micronutrients and the growth response compared to that in the unamended sample. The average concentration of soluble inorganic phosphorus (SIP) in March and June samples was 0.149 and 0.069 $\mu\text{g P/ml}$, respectively. The average algal available SIP in March and June samples was 0.094 and 0.031 $\mu\text{g P/ml}$, respectively (63 and 45% of SIP). The concentration of algal available P was not equal to or greater than the SIP concentration in any sample. The addition of micronutrients to water samples collected from the Maumee River in both March and June stimulated growth of *S. capricornutum*, suggesting that a micronutrient deficiency was limiting algal growth in the river water.

Predictability of Change in Soil Reflectance on Wetting. J. B. PETERSON, R. H. BECK, and B. F. ROBINSON, Laboratory for Applications of Remote Sensing, Purdue University, West Lafayette, Indiana 47907.——An array of ratios of the reflectances for oven-dry over those for 1/3-bar water levels at $0.708 \mu\text{m}$ of surface samples of 15 Indiana soils, 11 mollisols and 4 alfisols of which the mollisols (prairie soils) are darker colored by Munsell color standards and significantly higher in organic carbons than the alfisols (hard-wood forest soils),

shows two significantly different populations for the two groups by the T test. The mean ratio for the mollisols is 2.89 and for the alfisols 2.2.

However, when the decrease in reflectance at $.708\ \mu\text{m}$ resulting from wetness (oven-dry reflectance—1/3 bar reflectance) is evaluated over the entire 15 samples the regression shows an R^2 of .9350. The R^2 test for the loss in reflectance for the 11 mollisols, only, on wetting to 1/3 bar versus the reflectances when oven-dry increases to .97441.

When another reflectance band is used, the $1.94\text{--}2.06\ \mu\text{m}$ which is not greatly affected by atmospheric moisture when used with the Exotech-30, a similarly orderly relationship is apparent in the reduction in reflectance on increasing the moisture levels from oven-dry to 1/3 bar, in this case an R^2 of .9521.

These preliminary studies emphasize the very good possibility that a careful analysis over a larger sample of soils will show an orderly relationship in change of reflectance among different levels of wetness.

Effects of the Freeze-Thaw Cycles on Compacted Soils. GARY C. STEINHARDT, Agronomy Department, Purdue University, West Lafayette, Indiana 47907.——Soil compaction is a problem observed in many fields in Indiana. It is generally thought that the winter freezing and thawing has reduced soil compaction in the past. One inch deep cores of silt loam soil compacted by a hydraulic press were prepared. These cores were saturated then brought to a moisture content of 40 cm tension on a tension table. Strength of compaction was measured using a needle penetrometer. The cores were frozen in a -23°C freezer and thawed. Some of the cores had weights on top of the core to stimulate overlying soil material. The lowest strength was measured after the saturated core was brought to 40 cm tension not after freezing and thawing. The cores with weights on during the freezing and thawing had greater soil strength than those without weights.

A Determination of Amount of Suspended Solids Carried into Prairie Creek Reservoir. C. E. SPAID and H. F. SIEWERT, Department of Natural Resources, Ball State University, Muncie, Indiana 47306.——The amount of suspended matter carried by four major tributaries of Prairie Creek Reservoir were monitored from March to May, 1978. Positive correlations between discharge, suspended solids, and turbidity were observed. The average amount of suspended solids washed into the reservoir by the four tributaries was 1,379 kg/day during the spring. The life span of the reservoir was estimated.

Air Temperature Fluctuation Aboard the *Fairsea* During the Eclipse of 12 October 1977. WILLIAM R. GOMMEL and VIVIAN P. GOMMEL, Department of Earth Sciences, Indiana Central University, Indianapolis, Indiana 46227.——Using a Science Associates sling psychrometer in tropical waters 1375 nautical miles south of Los Angeles, California, a reduction in air temperature of approximately 4F was observed from 7 minutes after first contact (85.4F at 19:15 GMT) to the end of totality at third contact (81.4F at 20:46 GMT). Wet-bulb temperature fell 1.5F from 78.4F to 76.9F during the same period, and relative humidity increased from 73% to 81%. By 21:45 GMT, the temperature had increased to 84.8F.

At totality onset (12:08 PDT or 19:08 GMT) the ship was located at 11° 6.5'N, 118° 7'W and dead in the water. Clouds were 0.3 to 0.4 cumulus and 0.3 cirrus throughout the eclipse, and horizontal visibilities were more than 10 miles. Surface wind was SE 10-12 knots and did not seem to increase perceptibly as observed by the authors during the Norwegian and African eclipses of 30 June 1954 and 1973, respectively. Shadow bands (apparently an interference of light phenomenon) also were not as distinct as during the African eclipse of 30 June 1973.

The increased velocity of air flow at the observers' location in front of the ship's main air-intake screen resulted in an apparent adiabatic reduction in air temperature of approximately 0.5F (associated with the slight reduction in static pressure there). Otherwise, actual changes in the free air temperature over the ocean should be close to those observed by the authors. Under similar eclipse conditions over inland areas such as Indiana, air temperature fluctuations should be several times larger than observed over oceans.

Fate of Fertilizer Nitrogen Applied to Two Indiana Soils Cropped to Corn

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Introduction

Nitrogen is the plant nutrient applied in the largest quantities for crop production in the United States. On the average, Indiana corn producers apply higher amounts of N per unit area (130 kg/hectare/year) than farmers in other corn belt states. Corn yields typically increase with increasing N application rates up to about 200 kg/ha/year, however, little is known about the corn yield response to rates of applied N above 300 kg/ha. There is interest in applying large amounts of N to achieve high corn silage or grain yields. Cummings (1967) observed small corn yield increases with increased rate of applied N up to 896 kg/ha. However, Powell and Webb (1972) observed that corn yields decreased at N rates above 224 kg/ha on Webster clay loam soil at Ames, Iowa and above 448 kg/ha on another site of the same soil at Kanawha, Iowa.

There is little information on the ultimate fate of that portion of applied N which is not assimilated by plants, particularly where high N application rates are used. This information is needed because of possible water quality deterioration associated with the presence of large amounts of excess NO_3^- in the soil profile at the end of the cropping season. The application of large amounts of fertilizer N results in NO_3^- accumulation in the soil profile because corn plants can efficiently utilize only moderate amounts of N. Therefore, the objectives of the study reported here were to determine: (i) the effects of several N application rates (ranging from 0 to 1344 kg/ha/yr) on the yield of corn grown on two Indiana soils, and (ii) the fate of N over a four year period in two soils treated with varying rates of fertilizer N and cropped to corn.

Materials and Methods

Two soil types located approximately 1 km apart on the Purdue University Agronomy Farm near West Lafayette were selected for the study. One soil, a Chalmers silty clay loam (Typic Argioquall), was underlain by tile drains every 20 m and had the following characteristics at the surface: cation exchange capacity (CEC), 32.5 meq/100g; organic C, 2.2%; available P, 93 $\mu\text{g/g}$; and exchangeable K, 333 $\mu\text{g/g}$. The other soil, a Fincastle silt loam (Aeric Ochraqualf), was not tile drained (a limited amount of old field tile may have been present) and had the following characteristics: CEC, 16.3 meq/100 g; organic C, 1.115%; available P, 50 $\mu\text{g/g}$; and exchangeable K, 143 $\mu\text{g/g}$.

Plots (3 x 18.3 m) were established in the spring of 1969 using a randomized complete block design with four replications. Before fertilization the NH_4^+ , NO_3^- , and total-N concentrations in composite soil samples taken from the 0-15, 15-30, 30-60, 60-90, and 90-120 cm depths in each plots were determined. Urea

was broadcast at rates of 0,168,336,672, and 1344 kg N/ha in May and November 1969 and in November 1970 and 1971. Urea was incorporated by disking in May 1969 and by plowing when applied in November. The experimental areas were seeded to corn each year (Dekalb XL-45 in 1969 and Pioneer 3369A in 1970 thru 1972 in rows 76 cm apart and at a rate which resulted in an average population of 58,000 plants/ha at harvest. Weed and insect control was accomplished by common pesticides and by cultivation.

Steel access tubes 1 m in length with holes drilled near the lower end were placed in the center of each plot. Access tubes 3 m in length were placed in control plots and plots treated with 1344 kg N/year. Samples of the free water beneath the plots were withdrawn at various intervals over the four year period by use of a hand operated vacuum pump. The water samples were subsequently analyzed for NH_4^+ and NO_3^- -N by steam distillation (Bremner and Keeney, 1965).

At the end of each cropping year grain yield was determined by hand harvesting the middle 7.9 m section of the center two rows of each plot. Grain samples were dried, ground, and analyzed for total N (Nelson and Sommers, 1973). Each fall composite soil samples were taken from the 0-15, 15-30, 30-60, 60-90, and 90-120 cm depths of each plot. The samples were air-dried, crushed, and analyzed for NH_4^+ - and NO_3^- -N (Bremner and Keeney, 1966). At the end of the fourth cropping year, total N concentrations were also determined in each soil sample using the method of Nelson and Sommers (1972).

Nitrogen balances were calculated for the upper 1.2 m of each plot assuming a bulk density of 1.4 g/cm³ averaged over the profile. Bulk density measurements in a few plots confirmed the applicability of this value. Furthermore, even if the value assumed for bulk density is in error, comparisons between treatments would be valid because of the homogeneity in bulk density from plot to plot. Amounts of N added as fertilizer, present in the profile as NH_4^+ and NO_3^- at the start and end of the study, and removed as grain were accurately measured. Amounts of N added in rainfall and fixed by free-living microorganisms were estimated as 10 kg/ha/year. Net amounts of N mineralized during organic matter decomposition or immobilized during organic matter synthesis were estimated by changes in total N throughout the soil profile over the four year period. Nitrogen present in corn stover from the 1972 crop (this N would not be included in total N analysis of soil) was estimated from grain: stover ratios and N concentrations in stover observed in previous studies conducted on Chalmers and Fincastle soils.

All values reported are averages of four replications. All soil analysis data are expressed on a moisture-free basis and grain yields are calculated using a 15.5% moisture basis.

Results and Discussion

Table I presents data for corn yields as affected by rate of N applied to two soils over a four year period. During the first cropping year near maximum yields were obtained with application of 168 kg N/ha and yields tended to decline at higher N rates. The low yields obtained with 1344 kg N/ha may have resulted from accumulation of NH_3 or NO_2^- in soils shortly after urea application

TABLE I *Corn grain yields as affected by rates of nitrogen applied to two soils.*

Soil type*	N applied annually	Corn grain yield				
		1969	1970	1971	1972	Ave.
		kg/ha				
Chalmer sicl	0	7351	6423	2992	3776	5136
	168	8851	9628	11879	8649	9752
	336	8910	9697	12450	10223	10320
	672	8402	9044	12720	10029	10049
	1334	5111	9941	12695	10361	9527
Fincastle sil	0	7363	4453	1468	1555	3710
	168	7880	8950	9239	8894	8740
	336	7728	8840	10023	9897	9122
	672	5824	8222	8486	10825	8339
	1334	2562	9013	9245	11127	7987

*sicl, silty clay loam; sil, silt loam

and during seed germination. Both NH_3 and NO_2^- are phytotoxic when present in substantial amounts in the root zone. During subsequent cropping seasons maximum yields were obtained at N application rates of 168 or 336 kg/ha. The highest average yield over four years was obtained on both soil types in plots treated with 336 kg N/ha/year. On the average, N application rates higher than 336 kg/ha/year resulted in slightly decreased yields. The average yields on Chalmers and Fincastle soils obtained with 168 kg N/ha/year was 94.5% and 95.8%, respectively, of yields obtained with 336 kg N/ha/year. This finding suggests that maximum yields may have been obtained with a N application rate markedly below 336 kg N/ha/year. Other studies on these soils suggest that maximum corn yields may be obtained with from 200 to 230 kg N/ha/year.

The approximate N balance for Chalmers soil cropped to corn for a four year period is given in Table II. Net mineralization of N occurred at all N application rates except 1344 kg N/ha/year where about 1.5% of the added N

TABLE II *Approximate nitrogen balance sheet from Chalmers soil cropped to corn for four years.*

	Fertilizer N applied annually, kg/ha				
	0	168	336	672	1344
	kg N/ha				
N inputs:					
fertilizer	0	672	1344	2688	5376
mineralized	681	276	344	134	0
inorg. N. in profile	283	283	283	283	283
rainfall and fixation	40	40	40	40	40
Total	1004	1271	2011	3145	5699
N recovered:					
grain and stover	323	635	686	681	656
inorg. N in profile	106	131	133	241	464
immobilized	0	0	0	0	90
Total	429	766	819	814	1210
N lost from soil	575	504	1192	2331	4489

was incorporated into soil organic N. This finding suggests that very high N fertilizer additions would be required to increase the organic N content of Chalmers soils under continuous corn culture and that under normal fertilization practices significant amounts of organic N are mineralized each year (69 kg/ha/year at an N fertilizer rate of 168 kg/ha/year). The amounts of inorganic N recovered from the soil profile after four years were lower than those initially present for all N application rates except 1344 kg N/ha/year. This finding is surprising because it was presumed that inorganic N not used by plants would, in part, accumulate in the profile. Amounts of N recovered in grain plus stover increased with increasing N addition rate up to 336 kg/ha/year and then remained relatively constant.

The total amounts of N recovered in grain plus stover and inorganic and immobilized N in the soil profile after 4 years were much less than the amounts of N added to each plot. The proportion of N inputs which were recovered after 4 years was highest in plots receiving 168 kg N/ha/year (60.3%) and decreased in the following treatment order: 0>336>672>1344 kg N/ha/year. The proportion of added N recovered from plots receiving 1344 kg N/ha/year was only 21.2%.

The amounts of N apparently lost from Chalmers soil tended to increase with increasing rate of applied N except that N lost from plots treated with 168 kg N/ha/year was lower than that lost from untreated plots. This finding suggests that moderate fertilizer applications will not increase N loss from some soils and substantiates that N losses may be large (144 kg N/ha/year) from soils where no fertilizer was applied. Very large N losses were observed in plots receiving 672 and 1344 kg N/ha/year verifying that applications of N greatly in excess of plant needs will lead to poor recoveries of added N as a result of leaching/denitrification.

Table III presents an approximate N balance in Fincastle soil for a 4 year period. Net mineralization of organic N was observed in plots receiving 0 and 168 kg N/ha/year, whereas net immobilization of added N was obtained in plots

TABLE III *Approximate nitrogen balance sheet for Fincastle soil cropped to corn for four years.*

	Fertilizer N applied annually, kg/ha				
	0	168	336	672	1344
	 Kg N/ha				
N inputs:					
fertilizer	0	672	1344	2688	5376
mineralized	226	338	0	0	0
inorg. N in profile	206	206	206	206	206
rainfall and fixation	40	40	40	40	40
Total	472	1256	1590	2934	5622
N recovered:					
grain and stover	225	579	619	591	580
inorg. N in profile	34	66	172	596	706
immobilized	0	0	282	535	1355
Total	259	645	1073	1722	2641
N lost from soil	213	611	517	1212	2981

treated with > 336 kg N/ha/year. This finding suggests that the organic N content of Fincastle may be increased by applications of above average (~ 300 kg/ha/year) amounts of fertilizer N. Lower amounts of $(\text{NH}_4^+ + \text{NO}_3^-)\text{-N}$ were found after 4 years of cropping in plots receiving < 336 kg N/ha/year as compared to amounts of inorganic N in the soil profile at the start of the study. At N addition rates of > 672 kg/ha/year significant amounts of inorganic N accumulated in the profile. Amounts of N recovered in corn grain and fodder increased with increasing rate of fertilizer N up to 336 kg/ha/year and remained relatively constant at higher N application rates.

Total amounts of N recovered after 4 years increased with increasing rate of applied N. The proportion of added N which was recovered ranged from 67.5% in plots treated with 336 kg N/ha/year to 47% in plots receiving 1344 kg N/ha/year. Amounts of N apparently lost from the soil-plant system tended to increase with increasing rate of applied N except that higher N loss was observed in plots receiving 168 kg N/ha/year as compared to those treated with 336 kg N/ha/year. Average annual N loss ranged from 53 kg/ha for the unfertilized to 745 kg/ha for the plot receiving 1344 kg N/ha year.

It is interesting that the apparent N loss from Chalmers soil was larger than the loss from Fincastle soil at each N addition rate except 168 kg N/ha/year. This finding may result from the artificial drainage system installed in the Chalmers study area which provided a transport mechanisms for moving large amounts of $\text{NO}_3^- \text{-N}$ out of the soil profile. Determination of inorganic N in the free soil water at a depth of 1 m (approximate depth of drainage tile in Chalmers soil) provides direct evidence for $\text{NO}_3^- \text{-N}$ leaching. Fig. 1 and Fig. 2 give data for the $\text{NO}_3^- \text{-N}$ content of the soil water at the 1 m depth in Chalmers and Fincastle soils, respectively, treated with varying rates of fertilizer N. In Chalmers soils the $\text{NO}_3^- \text{-N}$ concentration in the soil water increased with N application rate at each sampling time except that data for the plots treated with 168 kg N/ha/year was similar to that of the unfertilized plot. There were large fluctuations in the $\text{NO}_3^- \text{-N}$ concentration in soil water with time in those plots treated with highest rates of N. Highest concentrations were normally measured in the winter and early spring. The fluctuations in $\text{NO}_3^- \text{-N}$ concentrations observed result from the varying amounts of water percolating through the soil profile during various seasons and the amounts of NO_3^- available for transport at the time of downward water movement.

More $\text{NO}_3^- \text{-N}$ tended to accumulate in the soil water below Fincastle plots as compared to Chalmers plots because free drainage by tiles was not provided in the Fincastle. Although the soil water from plots receiving 0 and 168 kg N/ha/year was low in $\text{NO}_3^- \text{-N}$, the soil water from plots treated with high rates of N had high $\text{NO}_3^- \text{-N}$ concentrations several times during the study. The $\text{NO}_3^- \text{-N}$ concentration in soil water tended to increase with increasing rate of N applied. There were extreme fluctuations in $\text{NO}_3^- \text{-N}$ concentrations of soil water with time in Fincastle soil. Highest $\text{NO}_3^- \text{-N}$ concentrations were in the winter and early spring following fall application of fertilizer. Further evidence for more NO_3^- accumulation in Fincastle soil as compared to Chalmers soil is provided by $\text{NO}_3^- \text{-N}$ analysis of the soil profile at the end of 4 cropping seasons (Table II and III) and by the higher corn yields obtained on Fincastle soil the first cropping season after N fertilization ceased.

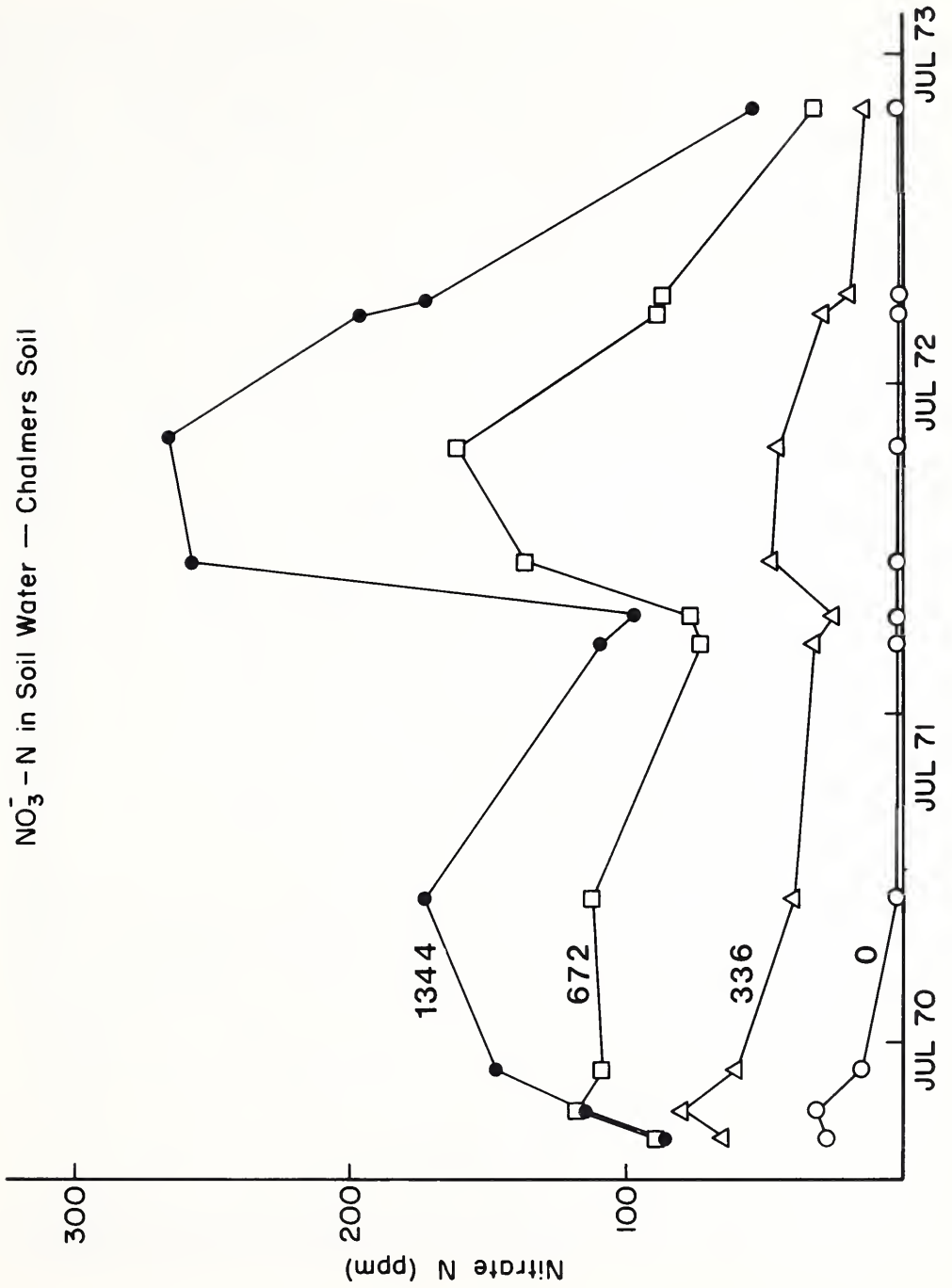


FIGURE 1. Nitrate N concentration in soil water at a depth of 1 m beneath Chalmers soil treated with varying rates of fertilizer nitrogen (numerical values above lines refer to rate of N applied in kg/ha/year.)

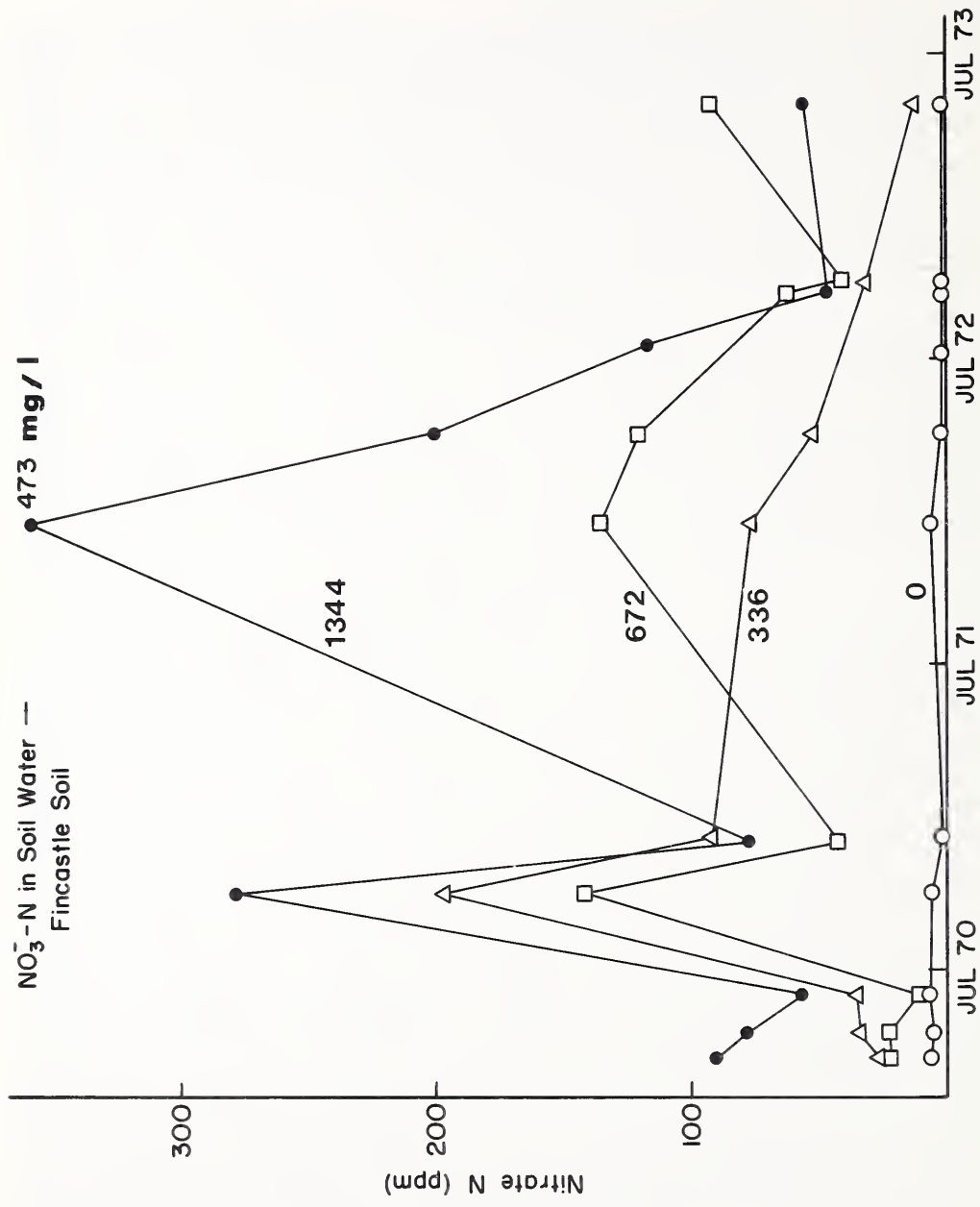


FIGURE 2. Nitrate N concentration in soil water at a depth of 1 m beneath Fincastle soil treated with varying rates of fertilizer nitrogen (numerical values above lines refer to rate of N applied in kg/ha/year).

Analysis of soil water from the 3 m depth established that the NO_3^- -N concentration was low in plots treated with 0 or 1344 kg N/ha/yr for both soil types. This finding suggests that NO_3^- -N is not being leached deep into the soil profile where the possibility for contamination of ground water supplies exist. However, it appears that water moving through tile drains is removing the excess NO_3^- -N from the lower portion of the soil profile. Analysis of the tile drainage water from the system draining the Chalmers study area indicated that NO_3^- -N concentrations were high (~ 25 mg NO_3^- -N/l).

Analysis of the profile of each soil at the end of the first cropping season indicated that substantial amounts of NO_3^- -N were present. However, the amounts of NO_3^- -N in the profile had decreased markedly by the start of the next cropping suggesting large over winter losses of N. It is unlikely that large amounts of NO_3^- -N was moved from the top of the soil profile to below 1.2 m by leaching during a 5 month period. Thus, it seems likely that denitrification in the early spring was partially responsible for NO_3^- -N losses from the soils studied.

The data collected in this study establish that high rates of fertilizer N (above 336 kg/ha/year) do not improve corn yields and result in extensive loss of N from the soil-plant system. The majority of the N lost from Chalmers and Fincastle soil is transported from the upper root zone by percolating water and is eventually removed in tile drainage water. A portion of the excess NO_3^- -N present in the profile at the end of the cropping season is apparently lost by denitrification in the early spring when soils are saturated.

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**Classification of the Sloping Soils
of the West Baden Group in Monroe County, Indiana**

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Introduction

There are over 15,500 acres of sloping soils formed on the West Baden Group (Chesterian Series, Mississippian System) in Monroe County, Indiana. The purpose of the field and laboratory study was to collect information that would help classify these soils according to the latest soil classification (8), to determine the composition of each landscape unit within the West Baden Group, and to show if any correlation between the landscape and soil series could be made. The study was also needed to determine which soils have a high shrink-swell potential, what causes the shrinking and swelling to occur, and what percentage of the landscape is made up of these high shrink-swell soils.

Soils that formed on the West Baden Group also occur in Crawford, Green, Harrison, Lawrence, Orange, Owen, and Washington Counties (2, 9). In those counties they were classified as Baxter, Berks, Corydon, Crider, Gilpin, and Wellston series in past surveys. In some areas they were mapped in complexes with the Berks, Gilpin, and Weikert series. In all the surveys, clayey soils with high shrink-swell potential have been considered inclusions within the mapping units that occur on the West Baden Group. All these soils developed under forest vegetation that consisted of oak, hickory, dogwood, tulip poplar, and cherry (4, 5, 10).

Geologic setting

Western Monroe County is a part of the Crawford Upland, the most rugged physiographic province in Indiana. Locally the relief exceeds 250 feet. The area has broad rounded ridgetops and broad valleys with gentle slopes. The most characteristic feature of the Crawford Upland is the great diversity of topographic forms produced by differing lithology and susceptibility to erosion of the underlying formations. The drainage is not always continuous because some streams disappear into sinkholes in the valleys.

Extensive areas in the Crawford Upland are underlain by the West Baden Group. This group is 100 to 140 feet thick and consists of the following formations in ascending order: Bethel Formation, Beaver Bend Limestone, Sample Formation, Reelsville Limestone, and Elwren Formation. The group consists primarily of gray and varicolored shale, sandstone, and discontinuous beds of limestone of variable thickness. Its predominantly clastic nature and the

irregularity of its limestone formations distinguish it from groups above and below (1, 6).

The West Baden Group is underlain conformably by a sequence of limestone formations, the Blue River Group (Valmeyeran Series, Mississippian System). It is overlain conformably by the Stephensport Group (Chesterian Series, Mississippian System) and, where the Stephensport Group has been removed by pre-Mansfield erosion, unconformably by the Mansfield Formation (Morrowan Series, Pennsylvanian System). The Stephensport Group includes about equal parts of limestone, gray shale, and thin-bedded to cliff-forming sandstone; the Mansfield Formation includes principally thin-bedded to cliff-forming sandstone and gray shale and is distinguished by scattered thin beds of coal (1, 6).

Method of study

A series of nine transects were made on sloping landscapes underlain by the West Baden Group. Detailed soil descriptions were made in representative areas wherever a change in the landscape occurred or within a distance of 100 to 175 feet. The soils were classified, their extent within the landscape unit was determined, and correlations were established between positions on the landscape and specific soils.

Two different kinds of landscape were distinguished on the basis of slope and soil composition. The major soil in both landscapes is a clayey soil that did not classify into existing soil series. To obtain additional data on the Ebal soil, a backhoe was used to expose a representative profile and the soil was sampled and described in detail. Bedrock at the site has been determined to be shale of the Sample Formation, West Baden Group (Henry H. Gray, personal communication, 1978).

Physical and chemical properties of the samples were determined at the Purdue Soil Characterization Laboratory, Purdue University, West Lafayette, Indiana 47907. Clay mineralogy was determined at the USDA Soil Conservation Service (SCS), National Soil Survey Laboratory, Lincoln, Nebraska. Standard laboratory procedures were used in these determinations (7).

Results and Discussion

Soil series on West Baden Group

Established soil series that have been mapped on formations of the West Baden Group include Baxter, Berks, Caneyville, Corydon, Crider, Gilpin, Hagerstown, Wellston, and Weikert series. The Ebal series is proposed as a result of this study.

The Baxter series is a fine, mixed, mesic Typic Paleudalf. It has less than 20 inches of loess over limestone residuum, and bedrock occurs at 60 to more than 120 inches. Chert content in the solum ranges from 15 to 35 percent.

The Berks series is a loamy-skeletal, mixed, mesic Typic Dystrochrept. It has sandstone, siltstone, or shale bedrock within 20 to 40 inches of the surface and contains more than 35 percent coarse fragments within the solum. It does not contain an argillic horizon.

The Caneyville series is a fine, mixed, mesic typic Hapludalf. It has limestone bedrock within 20 to 40 inches of the surface and contains up to 10 percent coarse fragments within the solum.

The Corydon series is a clayey, mixed, mesic Lithic Argiudoll. It has less than 20 inches of loess over limestone residuum and has limestone bedrock within 20 inches of the surface. It has a mollic epipedon and contains an argillic horizon.

The Crider series is a fine-silty, mixed, mesic Typic Paleudalf. It has more than 20 inches of loess over limestone residuum, and bedrock occurs at 60 to more than 120 inches.

The Frederick series is a clayey, kaolinitic, mesic typic Paleudult. It has limestone bedrock below 72 inches and the solum contains 5 to 15 percent coarse fragments. Its mineralogy is kaolinitic.

The Hagerstown series is a fine, mixed, mesic Typic Hapludalf. It has limestone bedrock within 40 to 100 inches of the surface and contains less than 5 percent coarse fragments within its solum.

The Gilpin series is a fine-loamy, mixed, mesic Typic Hapludult. It has sandstone, siltstone, or shale bedrock at 20 to 40 inches.

The Wellston series is a fine-silty, mixed, mesic Ultic Hapludalf. It has sandstone, siltstone, or shale bedrock at 40 to 72 inches.

The Weikert series is a loamy-skeletal, mixed, mesic Lithic Dystrochrept. It has sandstone, siltstone, or shale bedrock within 20 inches and contains more than 35 percent coarse fragments in its solum.

Proposed Ebal series

The proposed Ebal series is a fine, mixed, mesic Ultic Hapludalf. It is a deep, moderately well drained soil that formed in loess and residuum from interbedded shale and thin layers of sandstone. Permeability is moderate in the upper part of the solum and very slow in the lower part. Slopes range from 10 to 25 percent. This soil is a member of a large family, and no established soil series covers the range of characteristics of the proposed Ebal series. Therefore a new soil series is needed for these clayey shale soils.

The proposed Ebal soil has an argillic horizon with clay films on ped surfaces, which indicates clay movement, and slickensides are present in the IIIB24t horizon (appendix 1). The base saturation at 116 cm by the sum of cations is 55.1 (Table I). A weighted average of the particle size distribution in the control section places the soil in the fine textural class.

The mineralogy is mixed. The IIIB23t horizon has a moderate amount of extremely well crystallized kaolinite, montmorillonite, and mica. The upper part of the IIIB24t horizon contains a moderate amount of montmorillonite, well crystallized kaolinite, and mica; and the lower part contains a moderate amount of well crystallized kaolinite and mica and a small amount of montmorillonite and vermiculite. The presence of the montmorillonite clay in all three horizons explains the high shrink-swell potential.

TABLE I *Laboratory data for Ebal silt loam.*

Depth in cm	Horizon	Sand (2.0-0.05 mm)	Silt (0.05-0.002 mm)	Clay (<0.002 mm)	Coarse Fragments*	pH (1:1 H ₂ O)	Sum of cations	Base Saturation %	Texture class
0-7	A1	13.4	75.2	13.3	0	5.2	19.3	22.8	silt loam
7-20	B1	12.1	72.4	19.9	10	4.7	13.1	7.8	silt loam
20-33	B21t	17.0	55.2	27.8	23	5.0	14.2	12.2	silty clay loam
33-53	IIIB22t	20.5	33.7	45.8	38	5.0	24.1	21.4	clay
53-103	IIIB23t	8.5	30.3	61.2	0	4.7	31.8	27.1	clay
103-153	IIIB24t	5.6	31.0	63.4	0	4.1	30.8	38.9	clay
153-200	IIICr	37.7	28.7	33.6	0	3.8	55.4	72.4	clay loam
116	IIIB24t							55.1	

*Expressed as a percentage of all material at <76 mm.

Ebal series in soil map units

The major map unit on the West Baden Group makes up 10,175 acres in Monroe County. This map unit has been tentatively designated Ebal-Wellston-Gilpin complex, 12 to 18 percent slopes. It consists of strongly sloping, deep and moderately deep, moderately well and well drained soils on breaks that border the ridges of the upland. The unit contains about 50 percent Ebal soils, 20 percent Wellston soils, and 15 percent Gilpin soils. Berks soils are included throughout the unit and make up about 7 percent. On steeper sideslopes are small areas of Caneyville, Corydon, and Weikert soils. Most delineations contain only a few rock outcrops, bedrock escarpments, and short steep slopes. Throughout the unit are areas of Wellston and Gilpin soils that are so small in extent or so intricately mixed with Ebal soils that it was not practical to separate them in mapping. Wellston and Gilpin soils occur widely in south-central Indiana.

The minor soil map unit on the West Baden Group makes up 5,405 acres in Monroe County. This map unit has been tentatively designated Ebal-Gilpin-Hagerstown complex, 18 to 25 percent slopes. It consists of moderately steep, deep and moderately deep, moderately well and well drained soils on breaks that border the valleys and lower sideslopes of uplands. In the lower topographic positions, this unit formed on limestone of the Blue River Group (Henry H. Gray, personal communication, 1978). The unit contains about 50 percent Ebal soils, 30 percent Gilpin soils, and 16 percent Hagerstown soils. Wellston soils are included throughout the unit. On steeper sideslopes are small areas of Berks, Caneyville, Corydon, and Weikert soils. Most delineations have many large rock fragments on the surface and a few rock outcrops, bedrock escarpments, and short steep slopes. Throughout this unit are Gilpin and Hagerstown soils that are so small in extent or so mixed with the Ebal soils that it is not practical to separate them in mapping. This map unit contains more coarse fragments throughout the solum than the Ebal-Wellston-Gilpin complex. Ebal soils in the Ebal-Gilpin-Hagerstown complex would be taxadjuncts of the proposed Ebal series because they contain more coarse fragments than would be allowed for the proposed series. These soils are classified as clayey-skeletal, mixed, mesic Ultic Hapludalfs, whereas the proposed Ebal series is a fine, mixed, mesic Ultic Hapludalf.

In both map units, soils formed from limestone residuum are generally found only on the lower sideslopes that directly border the valley floors. In the Ebal-Wellston-Gilpin complex, limestone bedrock on the Blue river Group comes into contact with the overlying sandstone, siltstone, or shale in the lower third of the sideslopes. This limestone contact has generally been observed in the field as an eroded area, or slip, about 50 to 150 feet wide, where the limestone bedrock is exposed as rock ledges or outcrops. Soils directly below the limestone contact generally are formed in sandstone colluvium, but the underlying bedrock is limestone. In some areas the soils have formed in both the sandstone colluvium and limestone residuum, and in a few small areas the soils formed entirely in limestone residuum.

In the Ebal-Gilpin-Hagerstown map unit, the limestone contact occurs about halfway up the hillside and soils that formed entirely in limestone residuum are common on the lower part of most of the sideslopes.

In both landscape units, the soils above the limestone contact are intricately mixed because of the interbedding of sandstone, siltstone, and shale in the West Baden Group and because of strong colluvial movement of detached rock materials down the slope. The Ebal soils, which relate to shale bedrock, occur throughout the unit above the limestone contact. The position of the Ebal soils within the landscape depends upon where the strata of shale occur. In most cases, the Ebal soils are on small colluvium-covered benches directly below a short steep slope. This short steep slope usually contains sandstone bedrock exposures in the form of ledges and outcrops. The soils that occur directly below the Ebal soils are steeper than Ebal and formed in colluvium or residuum from sandstone.

Appendix 1

Profile description—Ebal silt loam

Location: Monroe County, Indiana—2,060 feet south and 920 feet east of the northwest corner, sec. 8, T. 7 N., R. 2 W.

Size of Area: 200 acres.

Vegetation: Hardwood forest.

Elevation: 770 feet above sea level.

Description:

- A1— 0-7 cm (0-3 in.), dark grayish brown (10YR 4/2) silt loam, light brownish gray (10YR 6/2) dry; moderate medium granular structure; friable; many fine, medium, and coarse roots; strongly acid; clear smooth boundary.
- B1— 7-20 cm (3-8 in.), yellowish brown (10YR 5/4) silt loam; weak medium subangular blocky structure; friable; many fine, medium, and coarse roots; 10 percent sandstone fragments, 3 percent greater than 2 mm (3/4 in.) in length; strongly acid; clear wavy boundary.
- B21t— 20-33 cm (8-13 in.), yellowish brown (10YR 5/14) channery silty clay loam; moderate medium subangular blocky structure; firm; common fine and medium roots; common discontinuous distinct thin yellowish brown (10YR 5/4) clay films on faces of peds; 23 percent sandstone fragments; very strongly acid; clear wavy boundary.
- IIB22t— 33-53 cm (13-21 in.), yellowish brown (10YR 5/4) very channery silty clay; moderate medium subangular blocky structure; firm; common fine and medium roots; common discontinuous distinct thin yellowish brown (10YR 5/4) clay films on faces of peds; 38 percent sandstone fragments; very strongly acid; clear wavy boundary.
- IIIB23t— 53-103 cm (21-41 in.), red (2.5YR 4/6) clay; many medium prominent gray (10YR 6/1) mottles; strong medium angular blocky structure; firm; few fine and medium roots; common discontinuous distinct medium gray (10YR 6/1) and yellowish brown (10YR 5/6) clay films on faces of peds; very strongly acid; clear wavy boundary.
- IIIB24t— 103-153 cm (41-61 in.), yellowish brown (10YR 5/4) clay; many coarse distinct gray (10YR 6/1) and strong brown (7.5YR 5/8)

mottles; strong medium angular blocky structure; firm; few fine and medium roots; common discontinuous distinct medium dark gray (10YR 4/1) and brown (10YR 5/3) slickensides on faces of peds; very strongly acid; gradual wavy boundary.

IIICr— 153-200 cm (61-80 in.), gray (10YR 6/1) clayey shale; many coarse distinct dark brown (7.5YR 4/4) mottles; very strongly acid.

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History of the Miami Series in Indiana

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Introduction

In 1900, when the Miami series was established, soil units were differentiated by surface texture alone. The term series had been borrowed from geology where it was used to define a time-stratigraphic group of sedimentary rock formations. The first definition of the term soil series was published in 1903 as "soils with a common feature, i.e., parent rock, and restricted to a physiographic province" (4). In that same year the Miami series was described as soils that were chiefly derived from morainic material of either glacial or alluvial origin" (4).

In 1904 soil survey field parties were advised that the Miami series was described as "light-colored sand or loam resting on light-colored glacial till or loess" and that it was restricted to areas north of the Ohio River (4). In 1906 the series was redefined as "light-colored upland timbered soils of glacial or loessial regions" (5). The color of the surface layer became a criterion for series differentiation, particularly for those soils in which the vegetational history greatly influenced soil color; therefore, many prairie areas previously mapped as the Miami series were distinguished as the Carrington series. The topographic setting was now required in series descriptions.

In 1914 the Miami series was redefined as soils with a "brown, light brown, or gray surface over yellowish-brown or darker subsoils, on nearly level to rolling topography, with fair to good drainage, on glacial drift parent material" (1). During this period, new criteria for series differentiation were added including, among other features, the organic matter content and reaction of surface soil and subsoil. Increasing attention was paid to describing drainage and to the identification of parent material.

In the early 1920's, soil began to be recognized as a natural body distinct from geological deposits. It was studied through the soil profile, and differentiating criteria were based on soil morphological features. New criteria introduced for series included the structure of the surface layer and subsoil and depth to carbonates. The Miami series description now included mention of a blocky structure in the subsoil and an acid reaction in the surface layer. The soils identified in the Miami series were all located in areas of calcareous parent material.

In the late 1920's and 1930's, new data greatly expanded our knowledge of how soils are formed. A new system of soil horizon designation, which was

indicative of soil genetic processes and behavior, was devised. Horizon thickness, consistency, and permeability were added to the list of series criteria.

The Soil Survey Manual of 1937 (2) defined a soil series as “a group of soils having genetic horizons similar as to differentiating characteristics and arrangement in the soil profile, and developed from a particular type of parent material. Except for texture, especially of the A horizon, the morphological features of the soil profile, as exhibited in the physical characteristics and thicknesses of the soil horizons, are now allowed to vary significantly within a series”. During the 1930’s and 1940’s, the Miami series concept was subjected to a few other minor changes and the parent material was restricted to the calcareous tills of the Wisconsin glacial stage.

In 1950 work began on a new soil classification system, one with a heavy emphasis on the characteristics of the subsoil. Changes in the Miami series definition during this period reflected the application of greater exactitude, including the adoption of standard soil color designations and inclusion of such indicators of soil genetic processes as clay coatings and color variations on and within peds. Family criteria, which separated soils with different mineralogy and restricted the textural range of the control section for a series, provided further standards for separation of soils in the Miami series from the 1950’s to the present.

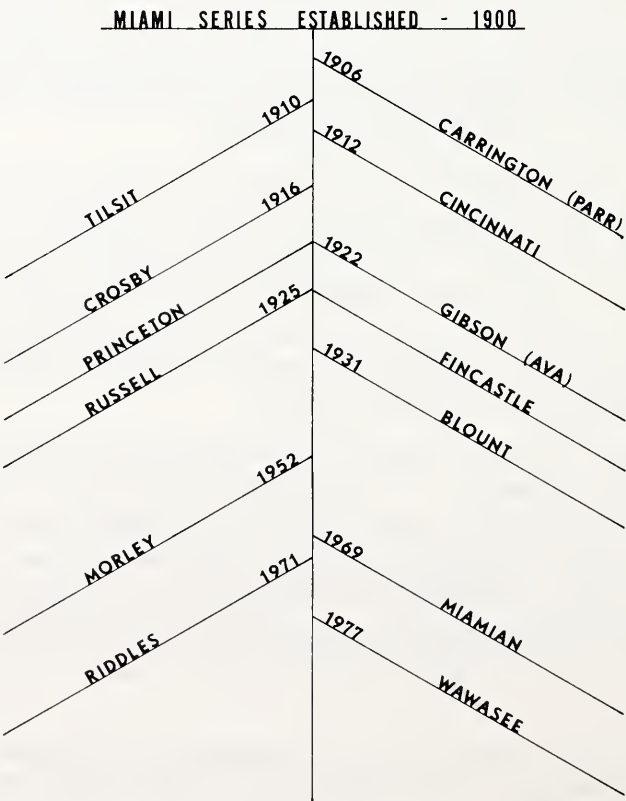


Fig. 1.- - Diagram showing major divisions of the Miami soil series, 1900 to 1978

Miami in Indiana

In Indiana, the major divisions of the Miami series and the times they were made are shown in (Fig. 1). Between 1900 and 1910, many of the upland soils in Indiana and some of the depressions were mapped in the Miami series. The reason for such wide use of one series at that time was the belief that soils were chiefly related to the broad classes of parent materials from which they formed. Most early soil scientists were trained as geologists and had little experience in studying the physical properties of soils or the genetic features responsible for their development.

It was not until much more was known about soils that soil features rather than simply the broad nature and physiography of the soil parent material became the main concern in defining the soil series.

Published reports during this period were very general and maps were broad and of small scale. Allen, Greene, Madison, Marion, Marshall, Newton, Posey, Scott, Tippecanoe, and Warrick (Boonville) were counties mapped during this period.

In the Madison County Soil Survey (published in 1903) Miami was conceived in a broad manner to represent 83 percent of the county area. Miami occurred on gently rolling and level areas of glacial gravelly clays. The soils were loams or clay loams grading to clay or clay loam subsoils. These extended from dissected land along streams across the broad interstream divides. Consequently, water ran off much of the land very slowly and large areas were somewhat poorly drained. Many of the dark level to depressional soils were poorly drained, and some of these were separated as Miami black clay loam.

Similar divisions were made of such geologic material in Newton, Marshall, and Tippecanoe Counties. In these counties, soils on deposits of wind-reworked sands were at least partially separated as Miami fine sand or Miami sand.

The Miami series was also characterized in Greene and other southern Indiana counties in a broad manner. The soils in the series were described as being developed in loess. The underlying till or bedrock was recognized but not considered to be a part of the soil. The soils were described as being silty and having more clay in the subsoil than in the surface. Flat areas had gray and mottled subsoils, and the soil on side slopes was yellow or reddish yellow. The description also pointed out the presence of a hardpan in some areas. We now recognize the hardpan layer as a fragipan.

In 1906 the Carrington series was established. This division recognized soils developed under prairie vegetation and had a dark surface layer high in organic matter. Those soils remaining in the Miami series had light colored surfaces and developed under forest vegetation.

In 1910 the Tilsit series was established. This division recognized soils that were associated with sandstone, siltstone, and shale and separated them from the Miami series.

In 1912 the Cincinnati series was established. This division separated the soils developed in loess capped Illinoian age till from the Miami series. After this, the Miami series was no longer used in surveys south of the south boundary of the Wisconsin age till.

In 1916 the somewhat poorly drained soils were separated from the Miami series and called Crosby. The Crosby series has since been separated into several series including Blount, Fincastle, and Crosier.

In 1922 in the Gibson County soil survey, the Princeton and Gibson (now Ava) series were established and used instead of the Miami series. The Gibson soils were soils that developed in loess where no carbonates were present. The Princeton series included soils that had carbonates in the loess parent material.

In 1925 in the survey of Wayne County, the Russell series was established. The Russell soils were till soils with a significant loess capping. This division left in the Miami series only the soils that developed in Wisconsin age till with thin to no loess capping. The Fincastle series was also established in this survey and was a division of the previously established Crosby series but also had a significant loess capping.

In 1952 the Morley series was established. This series separated the soils with silty clay loam and clay loam parent material from other soils of the Miami series, which had loam or sandy loam parent material. Morley soils also had a more clayey subsoil than Miami.

In 1969 the Miamian series was established. This division separated from the Miami series soils that have more than 35 percent clay in the textural control section (3) and yet overlie loam parent materials. The soils remaining in the Miami series were all in a fine-loamy family.

In 1971 the Riddles series was established. This soil had been recognized and separated from other Miami soils for several years, but a series had not previously been established. The Riddles series separated from the Miami series the soils that were leached of carbonates to more than 40 inches and had a solum more than 40 inches thick. Miami soils are now considered to have a solum less than 40 inches thick and carbonates within a depth of 40 inches.

In 1977 the Wawasee series was established. This division separated from the Miami series the soils that have less clay in the subsoil and are moderately permeable.

Miami Soils in 1978

At present the Miami series consists of deep, well drained soils with a moderately permeable subsoil and moderately slowly permeable underlying material. Miami soils formed in less than 18 inches of loess and in calcareous loam till. They are on uplands and have slopes of 0 to 30 percent.

Taxonomic class: Fine-loamy, mixed, mesic Typic Hapludalfs (3).

Typical Pedon

A1—0 to 3 inches; very dark grayish brown (10YR 3/2) silt loam; moderate medium and coarse granular structure; friable; slightly acid; clear wavy boundary. (1 to 3 inches thick)

A21—3 to 8 inches; brown (10YR 5/3) silt loam; moderate fine medium granular structure; friable; medium acid; clear smooth boundary. (0 to 7 inches thick)

A22—8 to 12 inches; yellowish brown (10YR 5/4) silt loam; weak medium platy structure; friable; medium acid; clear wavy boundary. (0 to 5 inches thick)

IIB21t—12 to 17 inches; dark brown (7.5YR 4/4) silty clay loam; moderate medium subangular blocky structure; friable; thin discontinuous pale brown (10YR 6/3) clay films on faces of peds; few pebbles; medium acid; clear smooth boundary. (4 to 7 inches thick)

IIB22t—17 to 25 inches; dark brown (10YR 4/3) clay loam; moderate medium and coarse angular block structure; firm; thin pale brown (10YR 6/3) and dark brown (7.5YR 4/4) continuous clay films on faces of peds and lining some voids; few pebbles; medium acid; clear wavy boundary. (4 to 10 inches thick)

IIB23t—25 to 29 inches; dark brown (10YR 4/3) loam; weak coarse subangular blocky structure; friable; few thin discontinuous dark brown (7.5YR 4/4) clay films on faces of peds and lining some voids; few pebbles; neutral; clear wavy boundary. (3 to 6 inches thick)

IIB3—29 to 36 inches; brown (10YR 5/3) loam; massive; friable; thin discontinuous dark brown (10YR 4/3) clay films; few pebbles; mildly alkaline; slight effervescence; clear irregular boundary. (0 to 8 inches thick)

IIC—36 to 60 inches; light yellowish brown (10YR 6/4) loam till; massive; firm; few pebbles; moderately alkaline; strong effervescence. Range in Characteristics:

Solum thickness 24 to 40 inches

Depth to carbonates less than 40 inches

Texture of surface silt loam or loam

Texture of subsoil typically clay loam with subhorizons which range from silty clay loam to loam

Texture of underlying material in loam

Conclusions

The soils remaining in the Miami series are extensive throughout central and northern Indiana. All divisions of the original Miami series have served a useful purpose. As the scientific knowledge about soils increased, differences among soils within the Miami series became easier to recognize, define and show on maps. Because increased uses of soil survey information required knowledge of soils to greater depths, the need for refinement of the series became more obvious.

As soil technology continues to develop and use of soil information continues to increase, perhaps more divisions of the Miami series will be made.

Acknowledgment

Historical information in the introduction was taken from material collected by George D. Bailey, Geographer, U.S. Department of Agriculture Soil Conservation Service, Glenn Dale, Maryland.

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**A Study of the Severity of Indiana's
1977 and 1978 Winters Using Heating Degree Days
Determined from Both Measured and Wind Chill Temperatures**

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Introduction

The past two winters have been distinctive in the severity of the weather experienced over the central United States. Indiana and its adjoining states were particularly hard hit. The objective of this study is to examine the severity of the winters of 1976-77 and 1977-78 in Indiana. The basic tool for this examination is the heating degree day, chosen because of its utility in portraying the cumulative effect of temperature conditions over a period of time.

While temperature itself (or the heating degree day) is a measure of the severity of a winter period, common experience teaches that the effect of temperature is influenced by the ventilating power of the wind. Thus, in order to incorporate this wind influence, a wind chill temperature is also calculated and used to determine a modified heating degree day. By combining the roles of both temperature and wind, a more comprehensive view of the severity of the two winters is achieved.

Included in the study are heating degree day and modified heating degree day conditions at six National Weather Service stations within and bounding Indiana: Indianapolis, Fort Wayne, South Bend, Evansville, Chicago (Midway), and Louisville.

The Winters of 1976-77 and 1977-78

The winter of 1976-77 was marked by severe cold in the central United States. In fact, the nation's greatest temperature anomaly occurred in the lower Great Lakes region, with Illinois, Indiana, and Ohio averaging 8° F or more below normal (11). Further, data published in (6) show that of the six stations used in this study, Evansville, Fort Wayne, Indianapolis, and Louisville listed January 1977 as the coldest January on record. January 1977 was also the coldest month on record at Evansville, Indianapolis, and Louisville. The atmospheric circulation pattern responsible for these conditions formed early in the fall and continued with little change until late February (11). Low pressure centers in the north Pacific Ocean and in eastern Canada were located south of their normal winter positions. Together with a strong ridge on the west coast, they brought extreme cold to the midwest and east coast (8). The strong ridge also resulted in the majority of the Pacific storms moving north into Alaska, leaving most of the United States with below normal precipitation. Indiana's precipitation was two to six inches below normal (12).

The winter of 1977-78 displayed an even greater departure from temperature normals in the Great Plains and Midwest (12). However, even though much of Indiana was about 9°F below normal for the winter, no new temperature records were set. Instead, new snowfall records were established. In January, South Bend, Chicago, and Louisville observed the greatest total monthly snowfall on record, while Evansville, South Bend, and Louisville recorded the greatest snowfall in 24 hours (6). The circulation pattern did not remain as constant as it did the previous winter. A deep trough, which had remained nearly stationary off the east coast in 1966-77, was located in various positions over the eastern United States in 1977-78 (12). Also, the southern portion of the west coast ridge was weaker than in 1976-77, which, when coupled with a strong subtropical jet stream, served to bring storms in from the Pacific ocean. After crossing the Rocky Mountains, these storms accumulated moisture from the Gulf of Mexico and then turned north, giving the eastern half of the country above normal amounts of precipitation (12). Indiana's precipitation was near normal, but a greater than normal amount was in the form of snowfall. The greatest snowfall anomaly in the nation was located around Lake Michigan with Indiana snowfall recorded at three to seven feet above normal (12).

The Heating Degree Day and Wind Chill Temperature

The primary tool used in this study was the heating degree (HDD). Simply stated, it is the departure of the daily average temperature ($\bar{T}$) from a reference temperature (T_R) (7), which in mathematical form becomes

$$\text{HDD} = \begin{cases} T_R - \bar{T} & \bar{T} < T_R \\ 0 & \bar{T} \geq T_R \end{cases} \quad (1)$$

The name of the variable is derived from its use in estimating winter heating fuel consumption, which is assumed to be zero when $\bar{T}$ equals or exceeds T_R . The heating degree day variable was used in the present study because it can be conveniently added for successive days to yield a quantity representative of the cumulative temperature conditions for a given winter period. The reference temperature used was 65°F, in accordance with National Weather Service practice (6).

The secondary tool used was the wind chill temperature. This is the hypothetical temperature that, when combined with a reference wind speed, produces the same amount of cooling as that produced by the observed temperature and wind speed. This cooling effect of temperature and wind speed was measured by Siple and Passel (9), members of the 1939-41 American Antarctic expedition. While in Antarctica, they performed over eighty experiments in subfreezing temperatures to determine the rate of freezing of a cylinder of water. Cooling rates in $\text{kg cal m}^{-2} \text{ hr}^{-1} \text{ } ^\circ\text{C}^{-1}$ were plotted against windspeed (v) in m s^{-1} . The resulting curve, together with an expression of the difference between neutral skin temperature (33°C) and ambient temperature (T) in $^\circ\text{C}$, was used to derive the empirical formula

$$K_o = (10v^{1/2} + 10.45 - v)(33 - T), \quad (2)$$

where K_o is the total dry-shade cooling measured in $\text{kg cal m}^{-2} \text{ hr}^{-1}$. Applying the

verbal definition stated above, an expression for wind chill temperature (T_w) can be derived from eq. (2) as

$$(10v^{1/2} + 10.45 - v)(33 - T) = (10V_R^{1/2} + 10.45 - V_R)(33 - T_w), \quad (3)$$

where V_R is the reference wind speed. Solving for T_w yields

$$T_w = \frac{(10v^{1/2} + 10.45 - v)(T - 33)}{(10V_R^{1/2} + 10.45 - V_R)} + 33 \bullet \quad (4)$$

The reference wind speed is generally considered to be the speed of air movement relative to a person walking on a calm day. This so-called walking wind speed is specified by Falconer (3) and Steadman (10) as 3 mi h^{-1} (approximately 1.3 ms^{-1}). Assuming this speed to be representative at a height of 1.5 m in a neutral stability atmosphere and applying a power law profile for the wind speed with the power set to $1/7$ (4), the wind speed at standard anemometer height (10m) would be 3.9 mi h^{-1} . This corresponds to the value of 4 mi h^{-1} used by the National Weather Service (13) in their calculation of T_w .

It should be mentioned that alternate formulas for K_o have been proposed. Court (2) believes different constants in Siple's formula give a better fit to the original data, while Steadman (10) proposes an entirely different formula that includes many additional physiological factors. Since this study is concerned with evaluating the severity of winter periods rather than evaluating their physiological effects, the inclusion of physiological factors other than those common to all of the formulas seemed unnecessary. Further, it is not within the scope of this paper to fully evaluate the various formulas. Since Siple's equation (eq. 4) is the original formulation and appears to be the most widely used (including by the National Weather Service (13)), it was the one chosen for this study.

Data and Computational Procedures

Data used in this study were derived from three NOAA/EDS publications (1, 5, 6). Daily mean temperature and wind speed data for the December through February, 1976-77 and 1977-78 winter periods (hereafter referred to as Winter 1977 and Winter 1978, respectively) were obtained from (6) for each of the six stations included in the study. To provide a basis for comparison against normal conditions, normal daily mean temperature data (1941-1970) were extracted from (1). Finally, normal wind speed data used were monthly mean normals for each station obtained from (5).

These data were used to calculate observed and normal daily and cumulative values of heating degree days (HDD) from eq. (1) and observed and normal daily average wind chill temperatures (T_w) from eq. (4). The latter were then inserted in eq. (1) to produce modified heating degree day (MHDD) values. To insure that the wind chill temperature would never be greater than the ambient temperature, T_w was set equal to T when the mean windspeed was less than 4 mi hr^{-1} (approximately 1.8 m s^{-1}).

Finally, to examine the extent to which the modified heating degree day variable gives a different impression of the severity of the winter than obtained

from the regular heating degree day, the following comparative ration was computed:

$$\text{RATIO} = (\text{MHDD} - \text{HDD})/(\text{MHDD}_n - \text{HDD}_n), \tag{5}$$

where the subscript n indicates normal values. A ratio value greater (less) than 1 indicates that the inclusion of wind data (through $\bar{T}_w$ and MHDD) suggests a more (less) severe winter than would be concluded without the wind data.

Results

Table 1 presents a summary of cumulative heating degree days, modified heating degree days, their normals, and the comparative ratio for the six stations in this study. In addition a six station average is included as an approximation to the state-wide average. HDD values for winter 1977 are typically 15 to 25% above normal, reflecting the severe cold experienced during this period. MHDD values are similarly well above normal. The comparative ratios, which are in general greater than one, indicate that when the effect of wind is included, the winter appears even more severe than it did on the basis of temperature data alone. In 1978, HDD values are slightly larger than in 1977. However, MHDD values are less than in 1977, reflecting weaker winds during the 1978 period. As a result of these lighter winds, the ratio is consistently less than one, indicating a less severe winter than revealed by temperature data alone. In comparing the two winters, the use of HDD and MHDD values leads to different conclusions regarding which winter was more severe. HDD values favor 1978, while MHDD values favor 1977. In other words, the inclusion of wind data does have some impact on evaluating the relative severity of the two winters.

TABLE 1 *Summary of heating degree days (HDD) and modified heating degree days (MHDD) for winter 1977 and 1978 presented with normals and comparative ratios.*

Station	Cumulative HDD	Cumulative HDD-normal	Cumulative MHDD	Cumulative MHDD-normal	Ratio
Winter 1977					
Chicago (Midway)	4141	3447	5906	4995	1.14
Evansville	3437	2739	4271	3845	.75
Fort Wayne	4153	3406	5998	4957	1.19
Indianapolis	3967	3167	5364	4559	1.00
Louisville	3197	2712	4396	3827	1.08
South Bend	3993	3495	5696	5115	1.05
Average	3814	3161	5271	4550	1.05
Winter 1978					
Chicago (Midway)	4105	3447	5604	4995	.97
Evansville	3575	2739	4540	3845	.87
Fort Wayne	4209	3406	5604	4959	.90
Indianapolis	3860	3167	5113	4559	.90
Louisville	3374	2712	4390	3827	.91
South Bend	4043	3495	5457	5115	.87
Average	3861	3161	5118	4550	.90

Distributions of cumulative HDD, MHDD, and their departures from normal are displayed in (Fig. 1), providing an overall view of the spatial relationships of HDD and MHDD for both winters. With the exception of Fort Wayne, both values exhibit a general north to south decrease. At Fort Wayne, more HDD and MHDD were accumulated and correspondingly larger departures from normal were recorded than at any of the other five stations. This indicates that the general latitude dependence of temperature can be interrupted during periods of extreme cold. The interrupting influence in this case could perhaps be the closer proximity of the two more northerly stations to Lake Michigan and/or to large metropolitan areas.

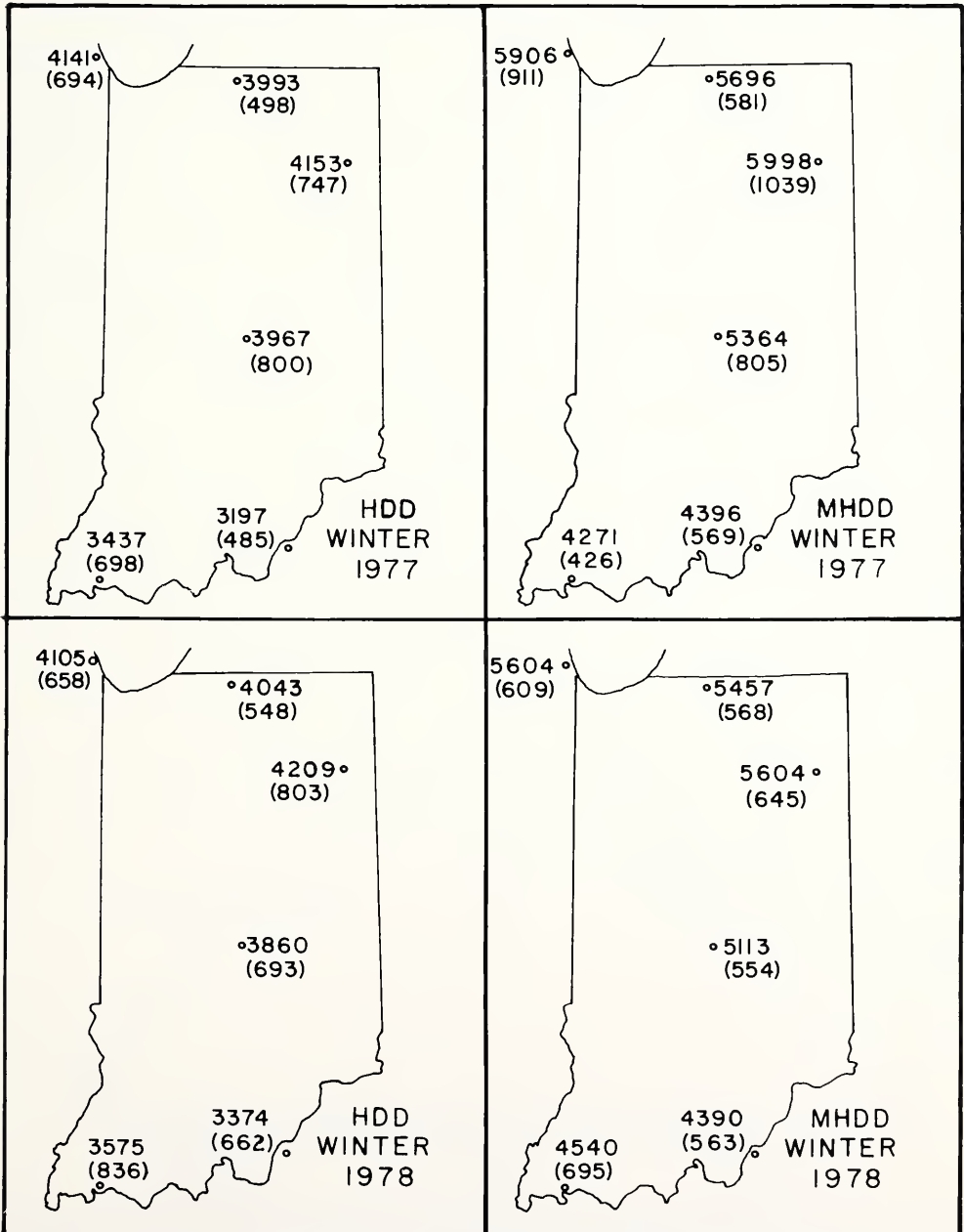


FIGURE 1. Cumulative heating degree days (HDD), modified heating degree days (MHDD) and departures from normal (in parentheses) for winter 1977 and 1978.

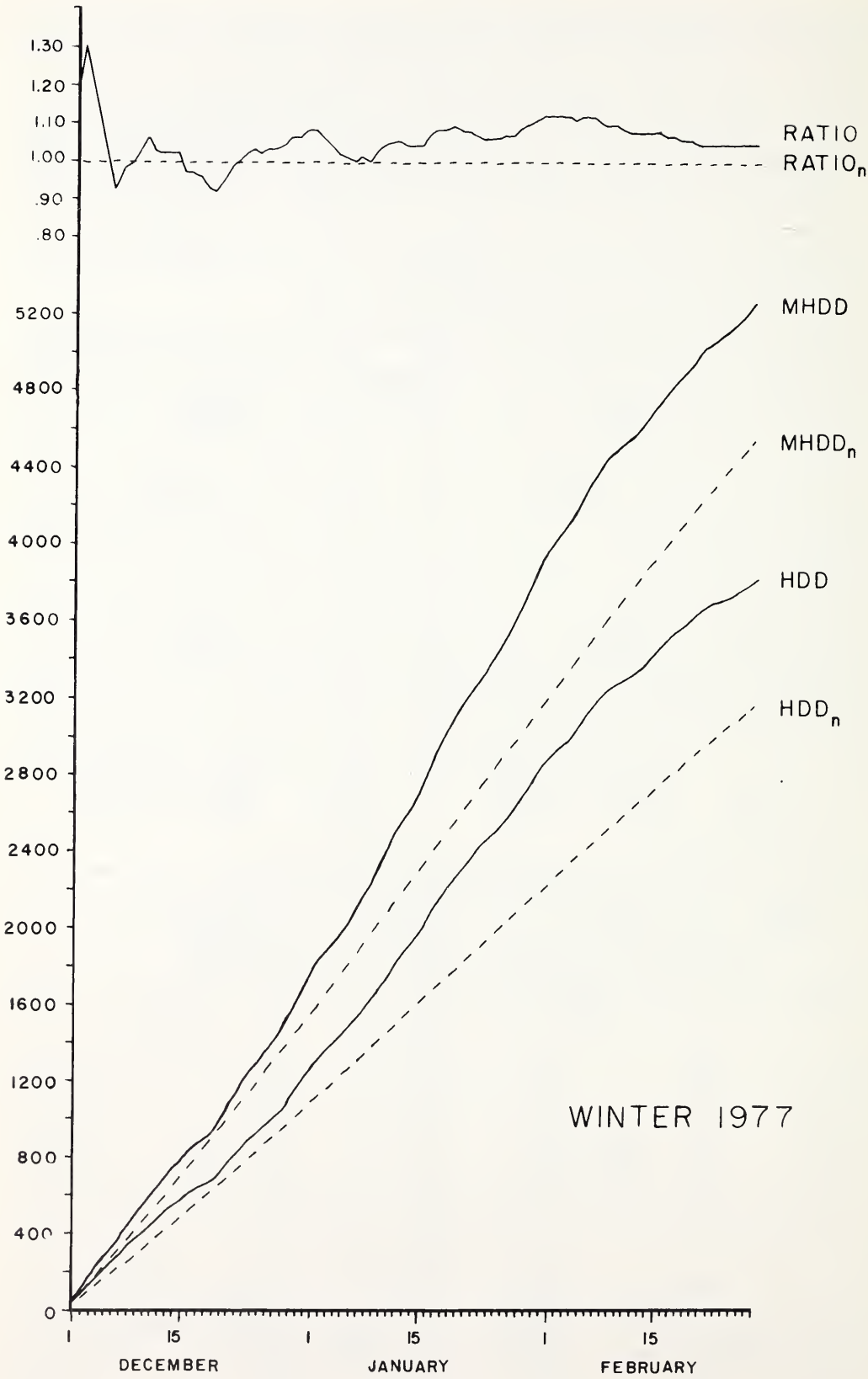


FIGURE 2. Daily cumulative heating degree days (HDD), modified heating degree days (MHDD), ratios (RATIO) and normals (indicated by subscript n) averaged over all stations for winter 1977.

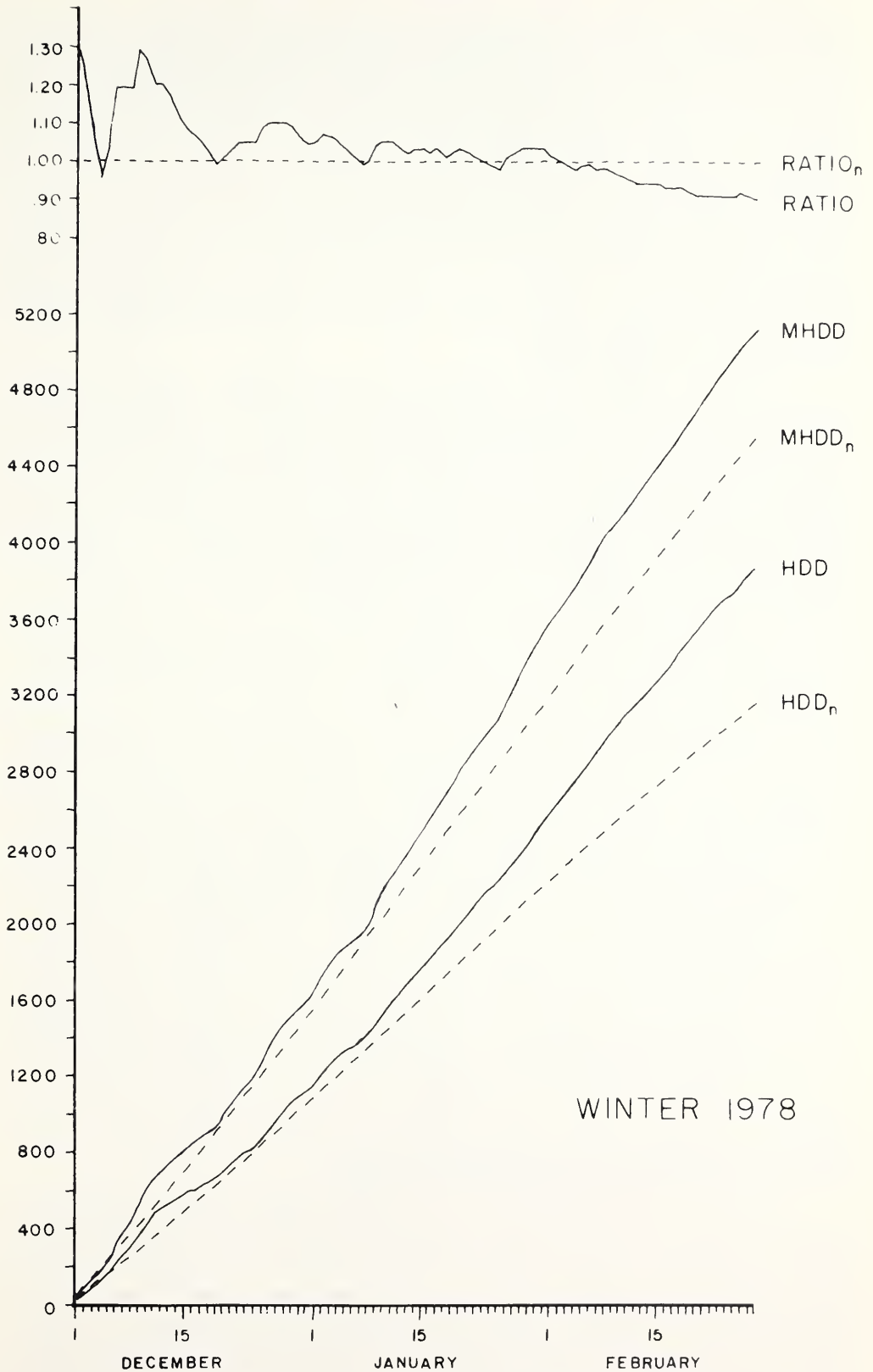


FIGURE 3. Daily cumulative heating degree days (HDD), modified heating degree days (MHDD), ratios (RATIO) and normals (indicated by subscript n) averaged over all stations for winter 1978.

(Fig. 2) and (Fig. 3) display daily cumulative HDD, MHDD, ratios and their normals averaged over all stations for winter 1977 and 1978, respectively. In 1977, both curves diverge from their normals as the winter progresses, with the maximum rate of divergence occurring from mid-January to early February, the period during which cold windy conditions were most prevalent. In December, the ratio values oscillate about 1.0, indicating that the wind had no consistent impact on the severity of the early part of the winter. However, from late December throughout the remaining winter period, the ratio is greater than 1.0, in response to the stronger than normal winds and the more rapid departure of MHDD and HDD from their respective normals. Thus, the cumulative effect of the winds throughout most of winter 1977 was to intensify the severity of the abnormally cold temperatures.

A similar examination of (Fig. 3) reveals that winter 1978 evolved in a somewhat different way than winter 1977. Both MHDD and HDD diverge from their normals at very steady rates from the second week in January through the end of February. However, after early January, the MHDD diverge at a slower rate than do the HDD. This is reflected in the ratio values. In the early part of the winter, oscillations in the ratio are present but rarely dip below 1.0, indicating that in contrast to winter 1977, the net effect of the wind was to increase the severity of the early winter. After the first week in January, the ratio gradually decreases, falling below 1.0 at the beginning of February. Thus, weaker than normal winds after early January reduced the potential severity of winter 1978.

Daily fluctuations of state average HDD, MHDD, and ratios for both winters are presented in (Fig. 4). Of the 90 total days in each winter period, between 70 and 75% experienced above normal HDD and MHDD values (colder than normal). It is interesting that both the maxima and minima on the MHDD curves are often amplified relative to the normal when compared to the same features on the HDD curves. This suggests that stronger (weaker) than normal winds often occurred on cold (warmer) than normal days and is reflected in a similar oscillating pattern in the ratio curves. In addition, the ratio curves show that the wind had its greatest impact in late December and January. The number of days in 1977 and 1978 with ratio values greater than 1.0 were 45 and 35, respectively. Thus, as seen in Table I and (Fig. 2) and (Fig. 3), winter 1977 was affected more significantly by wind than 1978. Comparing the 1977 and 1978 curves confirms that these two very cold winters evolved in quite different ways. Winter 1977 was marked by extreme cold in January followed by a greatly moderated February, while 1978 contained persistently colder than normal weather throughout both January and February.

In order to see the extent to which daily fluctuations vary between individual stations, the ratios for the two extreme stations, Chicago (Midway) and Evansville are shown in (Fig. 5). Chicago, the northernmost city in this study, had the most consistently large ratio for both years, while Evansville, the southernmost city, had the smallest ratio for both years (see Table I). Consistent with the state average, Chicago's 1977 ratio (1.14) was considerably larger than its 1978 ratio (.97). However, Evansville showed an increase from .75 in 1977 to .87 in 1978. The windy city clearly earned its nickname in 1977 with late December and January containing several maxima lasting four to seven days.

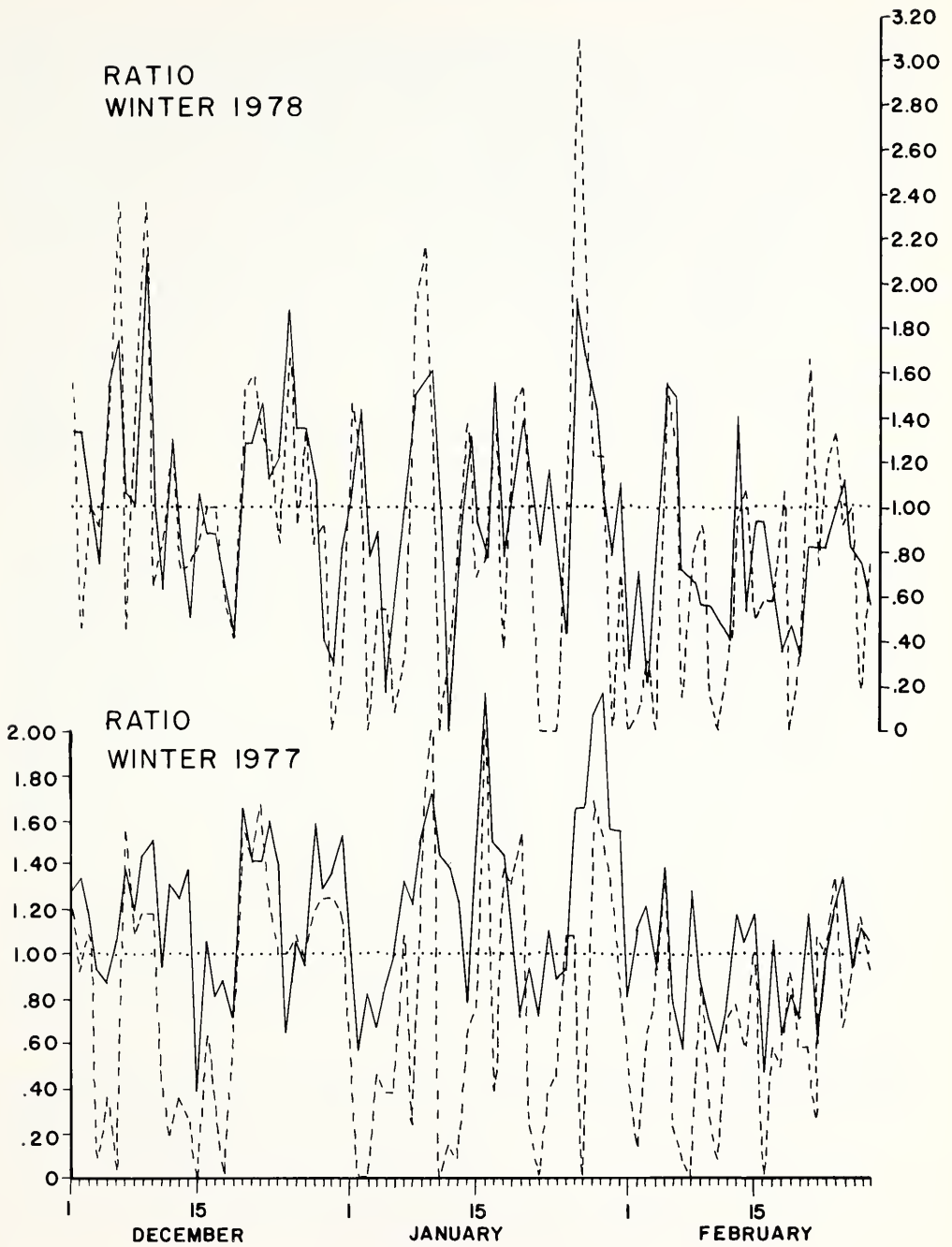


FIGURE 4. Daily heating degree days (HDD), modified heating degree days (MHDD), ratios (RATIO) and normals (dotted curves) for winter 1977 (solid curve) and winter 1978 (dashed curve).

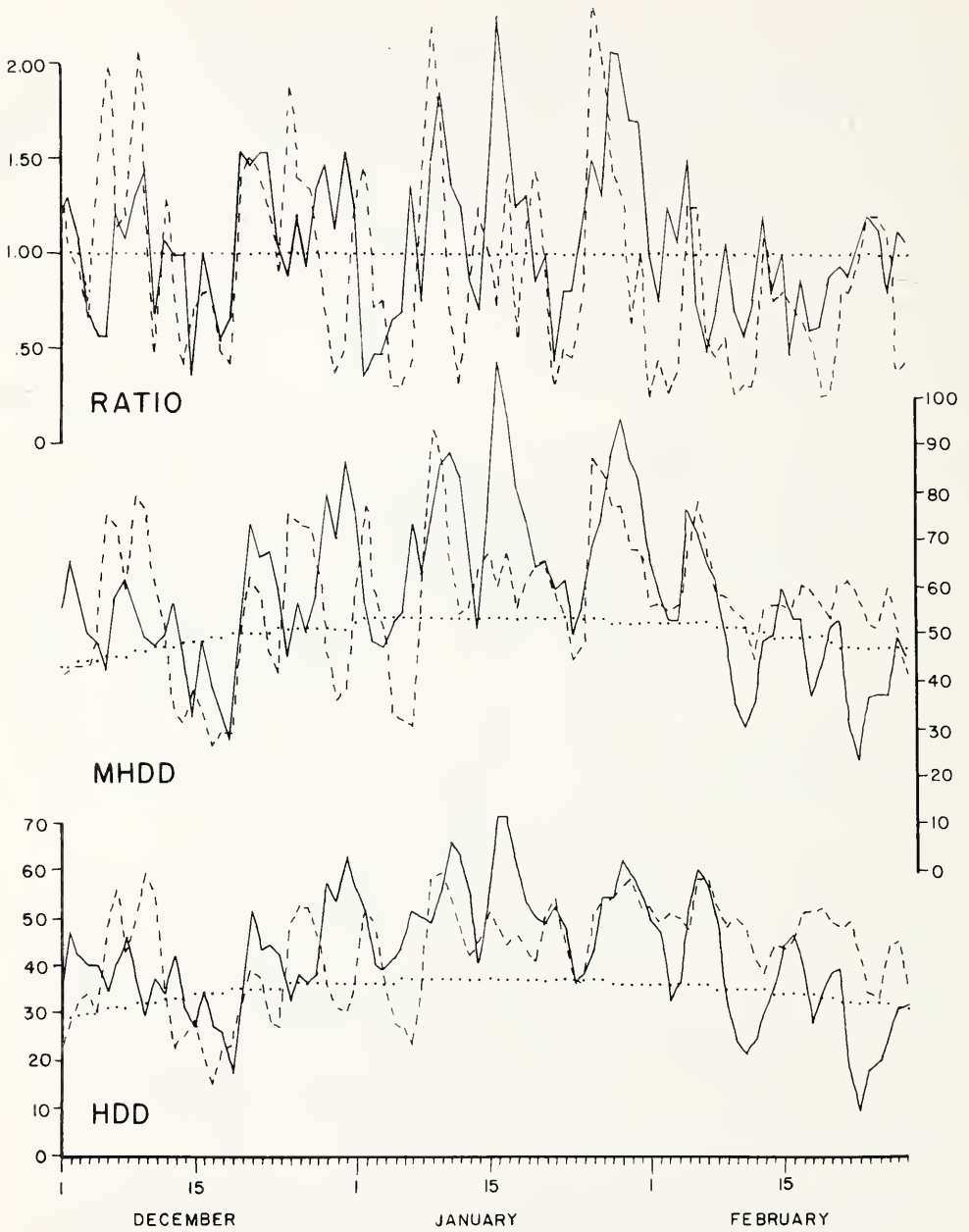


FIGURE 5. Daily ratios (RATIO) for Chicago (Midway) (solid curve), Evansville (dashed curve), and normal (dotted curve) for winter 1977 and 1978.

Above normal peaks tended to last three days or less at Evansville. In 1978, a larger number of significant fluctuations about the 1.0 line are seen at Chicago with December having more extreme and longer windy periods. In both years, Evansville showed a tendency not only for the winds to be less than normal, but also for their effect to frequently disappear. This happens only when the wind speed is less than the walking wind speed (reference wind speed) previously described. Over the total 180 day period, Chicago only recorded one day when the ratio was 0, while Evansville recorded 21. Even so, in 1978 Evansville had four cases of ratio values that exceeded 2.0 and coincidentally exceeded Chicago's ratio. For these cases, the effect of the wind is over twice its normal effect. For one of these cases, the ratio climbed to 3.08, the only instance encountered in this study in which the ratio exceeded 3.0. This occurred on January 26, the day of the Ohio valley blizzard of 1978.

Conclusions

The relative severity of the winters of 1977 and 1978 differs when the effects of wind and temperature are combined. When temperature alone is considered, as revealed by accumulated heating degree days, 1978 is seen to be just over 1% colder than 1977 for the six stations considered in this study. However, when wind is included, through a modified heating degree day based on the wind chill temperature, 1978 is about 3% "warmer" than 1977. While by any measure the two winters must be regarded as severe, the added influence of above normal wind speeds in 1977 amplified the impact of the cold temperatures, while lighter than normal winds in 1978 lessened the impact of similarly cold temperatures.

In addition, comparison of the two winters reveals the differing characteristics that severe winter periods can possess. In this study, one period was characterized by extreme temperature conditions in January followed by a period of moderating temperatures in February, while the other contained persistent cold throughout both January and February.

Finally, examination of individual stations reveals that most were significantly influenced by the wind in 1977 but experienced lighter than normal winds in 1978. However, in the southwestern part of the state, the lighter wind influence prevailed during both winters.

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ZOOLOGY

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Abstracts

Rabies in Bats From Indiana: 1973-1977. JOHN O. WHITAKER, JR., Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809, and LOUIS R. DOUGLAS, Indiana State Board of Health, 1330 West Michigan Street, Indianapolis, Indiana 46206. _____ A total of 642 bats from Indiana taken from 1973 through 1977 was examined for rabies, bringing to 1,957 the number examined for this virus since 1965. During this most recent five-year period, 15, or 2.3 percent, were rabid, as compared to 5.1 percent for the period 1968-1972. Of the 1,957, 82 were rabid (4.2 percent). The greatest number of cases was in red bats, *Lasiurus borealis*, and big brown bats, *Eptesicus fuscus*, but these were submitted in the greatest numbers. The greatest percentage infection occurred in the hoary bat, *Lasiurus cinereus*. Of 70 examined 13 were rabid (18.6 percent). Bats of the genera *Myotis*, *Lasionycteris* and *Nycticeius* were seldom rabid. Rabid bats were taken for the first time in DeKalb, Floyd, Fulton, Lake and Martin Counties during the 1973-77 period, bringing to 28 the number of counties from which bat rabies has been detected.

Serological Evidence of Arboviral Infections in East Central Indiana Wildlife, 1977-78. ROBERT R. PINGER, Public Health Entomology Laboratory, RALPH D. KIRKPATRICK and GLEN A. NELSON, Department of Biology, Ball State University, Muncie, Indiana 47306, MICHAEL J. SINSKO, Indiana State Board of Health, Indianapolis, Indiana 46206, PAUL R. GRIMSTAD, Laboratory for Arbovirus Research and Surveillance, Notre Dame University, South Bend, Indiana 46556, and DONALD C. DORSEY, Iowa State Hygienic Laboratory, University of Iowa, Iowa City, Iowa 52242. _____ Sera from 34 squirrels and rabbits collected in Delaware County were screened in microneutralization tests for antibodies against St. Louis encephalitis (SLE), western equine encephalomyelitis (WEE) and three viruses of the California encephalitis (CE) virus group, La Crosse (LAC), Trivittatus (TVT) and Jamestown Canyon (JC). Neutralizing antibodies to TVT virus were demonstrated in 8/13 red squirrels, 2/8 fox squirrels and 4/13 eastern cottontail rabbits. Antibodies to SLE virus were found in 4/14 mammalian sera from one collection site located within the city limits of Muncie, while none of the sera collected from a second site a mile outside the city were positive for SLE antibodies. However, serum from a single cottontail rabbit captured at the latter site neutralized WEE virus at a 1:8 dilution.

A total of 338 avian sera was collected from birds trapped and netted in Delaware and Grant Counties between June and September, 1978. These sera were tested for the presence of antibodies to SLE, WEE, and eastern equine encephalomyelitis (EEE) virus by the hemagglutination inhibition (HI) test. Two House Sparrows, one from Delaware County and one from Grant County, had low HI antibody titers (1:10) against SLE virus. None of the birds showed evidence of infection with WEE or EEE viruses.

Mebendazole Treatment of the Invasive Phase of Experimental Trichinosis in Mice. RICHARD O. MCCracken, Department of Biology, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana 46205. Recent reports have demonstrated the chemotherapeutic effectiveness of a benzimidazole anthelmintic, mebendazole, in the treatment of *Trichinella spiralis* infections in rats and mice. Because mebendazole (methyl 5-benzoyl-benzimidazole-2-carbamate) is used for the treatment of other helminthoses in man, and because it is highly active against enteral and parenteral *Trichinella* in laboratory animals, this compound is one of great potential utility in the treatment of trichinosis in man. However, comparatively few studies have been reported with mebendazole on the relative susceptibility of the parasite during the invasive phase of infection (days 14 to 21) of the efficacy of dosage regimens similar to those used in man. I report here observations on the efficacy of mebendazole against the invasive phase of trichinosis because of its special clinical importance. Mebendazole proved to be effective against the invasive phase of trichinosis in mice in a 3-day regimen at dosages close to, or within the range of those recommended by the manufacturer for the control of other helminthic infections in man. Trial of the drug in severe trichinosis in man should be considered.

The Effects of Dose and Age on the Uptake and Distribution of Vanadium-48 in the Albino Rat. DENNIS M. SOLLENBERGER and RICHARD J. VETTER, Bionucleonics Department, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907. Vanadium is an essential trace metal and is found widely throughout nature, but it is toxic and irritates the respiratory tract when encountered in large amounts. The effects of dose and age on the uptake and distribution of vanadium-48 in the albino rat were investigated in order to better understand the biological fate of vanadium and provide data to aid in setting safe environmental and occupational concentrations.

Vanadyl trichloride (VOCl_3) was administered intraperitoneally in various doses (0.0002, 1.0, 10, or 100 $\mu\text{g V}/100 \text{ g body weight}$) to 250-g rats or at a concentration of 10 $\mu\text{g}/100 \text{ g body weight}$ to rats in three different age groups. Each dose contained 5 $\mu\text{Ci } ^{48}\text{V}$. The rats were sacrificed at 1/4, 1, 4, or 16 days after injection. All major abdominal and thoracic organs and femur were collected along with a sample of blood and leg muscle. Urine and feces were collected daily from the animals sacrificed at 16 days. The tissues highest in ^{48}V concentration (% dose/g) at 16 days were, in descending order, femur, kidneys, spleen, liver, and testes. The fraction of the total dose deposited in the femur was independent of the administered dose while the fraction deposited in other tissues was inversely proportional to dose. The fraction excreted increased with

dose. As age increased, the fraction of administered dose deposited in tissues decreased and the fraction excreted increased.

A Description of Growth and Skeletal Differentiation During Chick Wing Development. MARY AGNES FADERAN and ROBERT J. STARK, Department of Biology, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana 46205.——The relationship between growth, cell division, and cartilage differentiation during early limb development has been the subject of numerous investigations, but little is known about the relationship between growth and ossification of the skeletal elements. We have examined the development of the chick wing from 5 days of incubation to hatching to determine if growth and ossification were related and what such a relationship would indicate about controlling mechanisms. Rates of growth (elongation and diameter), the time ossification was initiated and the rates of ossification were determined for each of the skeletal elements in the wing. Growth was exponential with the rates for all of the elements decreasing twice, first at 8.5-9.5 days and again at 16-16.5 days of development. Ossification of the diaphysis was observed to initiate in all the elements except the phalanges by 8-8.5 days of development and by 9-9.5 days in the phalanges. Epiphyseal ossification was initiated in all of the elements by 15.5 to 16 days of development. The rate of growth was observed to be directly proportional to the degree of ossification. The patterns of growth and ossification suggest the initiating factor(s) must be specific in point of action on each element but influence all the elements in unison.

Reestablishment of Retinal-Tectal Specificity During Goldfish Optic Nerve Regeneration. S. ROBERT HANES and ROBERT J. STARK, Department of Biology, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana 46205.——The temporal-spacial sequence of retinal ganglion cell differentiation during normal eye development is sufficient to establish a nerve cable which, if the axons retain their relative positions, would in its projection onto the tectum reflect the relative positions of the ganglion cells in the retina. We have examined the pattern of retrograde peroxidase transport to determine if a similar temporal-spacial sequence is established during optic nerve regeneration. The optic nerve was severed in 3-4 inch goldfish and horseradish peroxidase injected at the lesion. The eyes were removed at regular postoperative intervals, sectioned, and peroxidase stained. The cell bodies of ganglion cells at the nerve-retina junction were the first labeled, with progressively more cells labeled laterally with time. The labeling progressed at a constant rate of 0.175 mm/hr from the nerve toward the retinal margin. This results in approximately a 22 to 34 hour differential between when the peroxidase reached and ganglion cells near the nerve and those on the margin. When this differential is translated into the initiation of axon regeneration it is sufficient to reestablish a cable and the specificity of the tectal projection.

**Chiggers (Acarina: Trombiculidae)
From the Mammals of Indiana**

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Abstract

Twenty species of chiggers were recovered from 32 of 52 species of mammals examined from Indiana: *Chelodonta ouachitensis*, *Eutrombicula alfreddugesi*, *Euschoengastia hamiltoni*, *E. marmotae*, *E. ohioensis*, *E. peromysci*, *E. pipistrelli*, *E. setosa*, *E. trigenuala*, *Leptotrombidium myotis*, *Leptotrombidium peromysci*, *Microtrombicula trisetica*, *Miyatrombicula cynos*, *M. jonesae*, *Neotrombicula fitchi*, *N. lipovskyi*, *N. microti*, *N. sylvilagi*, *N. whartoni*, and *Walchia americana*.

Introduction

The purpose of this paper is to bring together available information on chiggers of wild mammals of Indiana, including new host and state records.

Materials and Methods

Chiggers for this study were collected by the senior author and his students during studies of mammals of Indiana by Russell E. Mumford (Purdue University) and John O. Whitaker, Jr. Larval chiggers were detected by scanning mammals with a 10 to 70 power zoom dissecting microscope, and were preserved in 70% alcohol. After transfer to Nesbitts solution containing acid fuchsin for relaxing and staining for 6 to 14 days, they were mounted in Hoyers solution. Cover slips were ringed with euparal.

Prior to published records from the present study, there were only two reports of chiggers from Indiana. Jenkins (1) named *Eutrombicula alfreddugesi* (Oudemans) from several counties in Indiana, and Nadchatram and Wilson (2) described *Mackiena (Tinpinia) migratoria* based on larvae from South Korea and from a bank swallow taken at Lafayette, Indiana.

Previous records of chiggers from Indiana mammals consist of the following: *Euschoengastia pipistrelli* from *Eptesicus fuscus*, *Pipistrellus subflavus*, *Myotis austroriparius*, and *M. lucifugus* (6) and on *M. keenii* (3); *Euschoengastia hamiltoni*, *Leptotrombidium myotis* and *Neotrombicula microti* on *Eptesicus fuscus* (3); *Euschoengastia peromysci*, *Neotrombicula whartoni*, *Eutrombicula alfreddugesi* and *Leptotrombidium* sp. from *Zapus hudsonius* (7); *Euschoengastia marmotae* and *Eutrombicula alfreddugesi* from *Marmota monax* (8); *Euschoengastia trigenuala* from *Scalopus aquaticus* (9); *Neotrombicula fitchi*, *Walchia americana*, *Neotrombicula sylvilagi*,

Miyatrombicula cynos, *Neotrombicula whartoni*, *Eutrombicula alfreddugesi*, *Leptotrombidium* sp. and *Microtrombicula trisetica* on squirrels of the genus *Sciurus* (10); and *E. alfreddugesi* from *Rattus norvegicus* (4), and the same chigger plus *Neotrombicula whartoni* from the opossum (5). Thus 15 species of chiggers including members of seven genera have been reported from Indiana mammals. This paper summarizes these and new records of chiggers from the fur of mammals of Indiana.

Results

Most (52) of the 54 species of native and feral mammals of Indiana have now been examined, and 33 species were infested with chiggers. Neither big-eared bats, *Plecotus rafinesquii*, nor bobcats, *Lynx rufus*, were examined, and the following species of mammals lacked chiggers (number examined is in parentheses): INSECTIVORA: *Sorex longirostris* (13), *Blarina brevicauda* (133), *Cryptotis parva* (108), *Condylura cristata* (21). CHIROPTERA: *Myotis grisescens* (1), *M. sodalis* (43), *Lasionycteris noctivagans* (8), *Nycticeius humeralis* (12), *Lasiurus borealis* (234), *L. cinereus* (25). RODENTIA: *Spermophilus tridecemlineatus* (123), *S. franklinii* (14), *Geomys bursarius* (85), *Castor canadensis* (2), *Reithrodontomys megalotis* (80), *Mus musculus* (475). CARNIVORA: *Canis latrans* (15), *Taxidea taxus* (11). ARTIODACTYLA: *Odocoileus virginianus* (4).

Mammals with chiggers are listed in Table I, along with chiggers and relative abundance. The species of chiggers are listed in Table II, together with the hosts for each. A total of 20 species of chiggers, representing eight genera, have been found on mammals of Indiana. All of the records included here have resulted from the present series of studies, and 5 of these species are reported for the first time from the state, along with many new host records.

One species, *Eutrombicula alfreddugesi*, was widespread on Indiana mammals, being found on thirteen different hosts, but was not abundant on any. Usually reptiles are its major host. *Neotrombicula whartoni* was found on the same number of hosts, 13, but showed certain host affinities, particularly the cottontail rabbit, opossum and fox squirrel. A closely related species, *Neotrombicula lipovskyi*, also occurred on four hosts: two rodents, a carnivore and a rabbit.

Three of the species are bat chiggers, *Euschoengastia pipistrelli*, *E. hamiltoni* and *Leptotrombidium myotis*. The first was the only species found on bats of the genera *Myotis* and *Pipistrellus*, whereas all three species were found on *Eptesicus fuscus*, the big brown bat. However, on this latter species, *E. hamiltoni* was the most abundant chigger.

Three of the species could be termed squirrel chiggers in Indiana, particularly *Neotrombicula fitchii*, the most abundant chigger of all species of tree squirrels (*Glaucomys*, *Sciurus*, and *Tamiasciurus*), but also *Walchia americana* (primarily on *Sciurus*) and *Leptotrombidium peromysci*.

Three species of *Euschoengastia* are primarily rodent (other than squirrel) chiggers, but each had primary hosts. These species, with their primary hosts, were *E. peromysci* on the bog lemming and to a lesser degree on the white-footed

TABLE 1 *Chiggers of Wild Mammals of Indiana.*

Chigger Species	Number of Chiggers		Hosts Infested	
	Total	X	No.	Percent
MARSUPIALIA				
<i>Didelphis virginiana</i> , Virginia opossum (66 examined)				
<i>Neotrombicula whartoni</i>	137	2.08	14	21.2
<i>Eutrombicula alfreddugesi</i>	2	0.03	1	1.5
INSECTIVORA				
<i>Sorex cinereus</i> , Masked shrew (40 examined)				
<i>Euschoengastia ohioensis</i>	11	0.28	1	2.5
<i>Scalopus aquaticus</i> , Eastern mole (104 examined)				
<i>Euschoengastia trigenuala</i>	3	0.03	1	1.0
CHIROPTERA				
<i>Myotis austroriparius</i> , Southeastern myotis (5 examined)				
<i>Euschoengastia pipistrelli</i>	1	0.20	1	20.0
<i>M. keenii</i> , Keen's myotis (33 examined)				
<i>Euschoengastia pipistrelli</i>	48	1.45	9	27.3
<i>M. lucifugus</i> , Little brown myotis (84 examined)				
<i>Euschoengastia pipistrelli</i>	109	1.30	20	23.8
<i>Pipistrellus subflavus</i> , Eastern pipistrelle (56 examined)				
<i>Euschoengastia pipistrelli</i>	54	0.96	13	23.2
<i>Eptesicus fuscus</i> , Big brown bat (491 examined)				
<i>Euschoengastia hamiltoni</i>	34	0.1	5	1.0
<i>Leptotrombidium myotis</i>	7	0.01	4	0.8
<i>Euschoengastia pipistrelli</i>	3	0.01	2	0.4
<i>Neotrombicula microti</i>	1	0.002	1	0.2
LAGOMORPHA				
<i>Sylvilagus floridanus</i> , Eastern cottontail (43 examined)				
<i>Neotrombicula whartoni</i>	159	3.70	14	32.6
<i>Euschoengastia setosa</i>	3	0.07	2	4.7
<i>Neotrombicula lipovskyi</i>	1	0.02	1	2.3
RODENTIA				
<i>Sciurus carolinensis</i> , Gray squirrel (47 examined)				
<i>Leptotrombidium peromysci</i>	14	0.30	3	6.4
<i>Neotrombicula fitchi</i>	13	0.28	8	17.0
<i>Walchia americana</i>	13	0.28	6	12.8
<i>Neotrombicula whartoni</i>	10	0.21	3	6.4
<i>Eutrombicula alfreddugesi</i>	4	0.09	4	8.5
<i>Microtrombicula trisetica</i>	1	0.02	1	2.1
<i>Sciurus niger</i> , Fox squirrel (137 examined)				
<i>Neotrombicula whartoni</i>	68	0.50	9	6.6
<i>Walchia americana</i>	65	0.47	8	5.8
<i>Neotrombicula fitchi</i>	47	0.34	15	10.9
<i>Neotrombicula sylvilagi</i>	5	0.04	1	0.7
<i>Eutrombicula alfreddugesi</i>	4	0.03	3	2.2
<i>Leptotrombidium peromysci</i>	4	0.03	2	1.5

TABLE I Chiggers of Wild Mammals of Indiana. Continued

Chigger Species	Number of Chiggers		Hosts Infested	
	Total	X	No.	Percent
<i>Miyatrombicula cynos</i>	2	0.01	2	1.5
<i>Tamiasciurus hudsonicus</i> , Red squirrel (48 examined)				
? <i>Miyatrombicula cynos</i>	1	0.02	1	2.1
<i>Neotrombicula fitchi</i>	28	0.58	1	2.1
<i>Leptotrombidium peromysci</i>	2	0.04	1	2.1
<i>Marmota monax</i> , Woodchuck (91 examined)				
<i>Euschoengastia marmotae</i>	131	1.44	5	5.5
<i>Eutrombicula alfreddugesi</i>	12	0.13	1	1.1
<i>Euschoengastia peromysci</i>	2	0.02	1	1.1
<i>Tamias striatus</i> , Eastern chipmunk (65 examined)				
<i>Neotrombicula whartoni</i>	23	0.35	7	10.8
<i>Eutrombicula alfreddugesi</i>	3	0.05	1	1.5
<i>Euschoengastia peromysci</i>	2	0.03	1	1.5
<i>Walchia americana</i>	1	0.02	1	1.5
<i>Glaucomys volans</i> , Southern flying squirrel (11 examined)				
<i>Neotrombicula fitchi</i>	40	3.64	1	9.1
<i>Peromyscus maniculatus</i> , Deermouse (454 examined)				
<i>Euschoengastia peromysci</i>	1	0.002	1	0.2
<i>Peromyscus leucopus</i> , White-footed mouse (209 examined)				
<i>Euschoengastia peromysci</i>	63	0.30	11	5.3
<i>Euschoengastia setosa</i>	1	0.004	1	0.5
<i>Miyatrombicula jonesae</i>	1	0.004	1	0.5
<i>Neotoma floridana</i> , Eastern woodrat (10 examined)				
<i>Euschoengastia setosa</i>	55	5.50	6	60.0
<i>Neotrombicula lipovskyi</i>	44	4.40	3	30.0
<i>Euschoengastia peromysci</i>	2	0.20	1	10.0
<i>Cheladonta ouachitensis</i>	1	0.10	1	10.0
<i>Synaptomys cooperi</i> , Southern bog lemming (25 examined)				
<i>Euschoengastia peromysci</i>	299	11.96	24	96.0
<i>Euschoengastia ohioensis</i>	26	1.04	8	32.0
<i>Eutrombicula alfreddugesi</i>	8	0.32	1	4.0
<i>Euschoengastia setosa</i>	5	0.20	1	4.0
<i>Ondatra zibethicus</i> , Muskrat (31 examined)				
<i>Euschoengastia peromysci</i>	1	0.03	1	3.2
<i>Microtus ochrogaster</i> , Prairie vole (32 examined)				
<i>Euschoengastia setosa</i>	11	0.34	3	9.3
<i>Eutrombicula alfreddugesi</i>	5	0.16	4	12.5
<i>Neotrombicula whartoni</i>	1	0.03	1	3.1
<i>Microtus pennsylvanicus</i> , Meadow vole (82 examined)				
<i>Euschoengastia peromysci</i>	39	0.48	5	6.1
<i>Neotrombicula whartoni</i>	1	0.01	1	1.2

TABLE I Chiggers of Wild Mammals of Indiana. Continued

Chigger Species	Number of Chiggers		Hosts Infested	
	Total	X	No.	Percent
<i>Neotrombicula lipovskyi</i>	1	0.01	1	1.2
<i>Eutrombicula alfreddugesi</i>	1	0.01	1	1.2
<i>Microtus pinetorum</i> , Pine vole (27 examined)				
<i>Euschoengastia ohioensis</i>	69	2.55	5	18.5
<i>Euschoengastia peromysci</i>	13	0.48	1	3.7
<i>Neotrombicula lipovskyi</i>	3	0.11	1	3.7
<i>Zapus hudsonius</i> , Meadow jumping mouse (115 examined)				
<i>Euschoengastia peromysci</i>	12	0.10	2	1.7
<i>Neotrombicula whartoni</i>	5	0.04	1	0.8
<i>Eutrombicula alfreddugesi</i>	3	0.03	2	1.7
<i>Leptotrombidium peromysci</i>	1	0.01	1	0.8
<i>Rattus norvegicus</i> , Norway rat (146 examined)				
<i>Eutrombicula alfreddugesi</i>	1	0.01	1	0.7
CARNIVORA				
<i>Vulpes vulpes</i> , Red fox (14 examined)				
<i>Neotrombicula whartoni</i>	40	2.86	1	7.1
<i>Miyatrombicula jonesae</i>	6	0.43	1	7.1
<i>Urocyon cinereoargenteus</i> , Gray fox (42 examined)				
<i>Neotrombicula lipovskyi</i>	150	3.57	1	2.4
<i>Neotrombicula whartoni</i>	1	0.02	1	2.4
<i>Canis latrans</i> , Coyote (15 examined)				
<i>Eutrombicula alfreddugesi</i>	9	0.60	1	6.7
<i>Procyon lotor</i> , Raccoon (54 examined)				
<i>Neotrombicula whartoni</i>	5	0.09	1	1.9
<i>Neotrombicula microti</i>	4	0.07	1	1.9
<i>Mustela frenata</i> , Long-tailed weasel (16 examined)				
<i>Eutrombicula alfreddugesi</i>	7	0.44	2	12.5
<i>Euschoengastia peromysci</i>	1	0.06	1	6.3
<i>Mustela nivalis</i> , Least weasel (8 examined)				
<i>Euschoengastia peromysci</i>	1	0.12	1	12.5
<i>Mustela vison</i> , Mink (12 examined)				
<i>Neotrombicula whartoni</i>	2	0.17	2	16.7
<i>Mephitis mephitis</i> , Striped skunk (10 examined)				
<i>Neotrombicula whartoni</i>	1	0.10	1	10.0

mouse; *E. ohioensis* on the pine vole; and *E. setosa* on the woodrat and meadow vole.

Seven species of chiggers were found on only one species of host, *Euschoengastia marmotae*, with 131 individuals on the woodchuck, *E.*

hamiltoni and *Leptotrombidium myotis* with 7 and 3 individuals on the big brown bat, *Neotrombicula sylvilagi* with 15 individuals on the fox squirrel, *Microtrombicula trisetica* with one individual on the gray squirrel, *Euschoengastia trigenuala* with 3 individuals on the Eastern mole, and a single larva of *Cheladonta ouachitensis* from a wood rat. Only the first two of these relationships were supported with adequate data to suggest host-specific relationships.

Many of the species of chiggers tend to be either warm or cold weather chiggers, and this was well illustrated during the present study (Table II). The warm weather chigger *Eutrombicula alfreddugesi* is one of the common pest chiggers of humans throughout the summer in the midwest. The remainder are cold weather chiggers, except for a few species such as the bat-infesting *Euschoengastia pipistrelli*, and those inadequately studied.

TABLE II Numbers of chiggers taken from the various species of wild mammals of Indiana, summarized bimonthly. (For numbers of hosts examined, see Table I.)

Host Species	Dec.- Jan.	Feb.- March	April- May	June- July	Aug.- Sept.	Oct.- Nov.	Totals
<i>Cheladonta ouachitensis</i>							
Lipovsky, Crossley & Loomis, 1955							
<i>Neotoma floridana</i>						1	1
<i>Eutrombicula alfreddugesi</i> (Oudemans, 1910)							
<i>Marmota monax</i>				12			12
<i>Synaptomys cooperi</i>				8			8
<i>Mustela frenata</i>				1	6		7
<i>Microtus ochrogaster</i>				5			5
<i>Sciurus niger</i>					4		4
<i>Sciurus carolinensis</i>					4		4
<i>Tamias striatus</i>					3		3
<i>Zapus hudsonius</i>				2	1		3
<i>Didelphis virginiana</i>					2		2
<i>Microtus pennsylvanicus</i>				1			1
<i>Urocyon cinereoargenteus</i>				1			1
<i>Canis latrans</i>					9		9
<i>Rattus norvegicus</i>						1	1
<i>Euschoengastia hamiltoni</i> Brennan, 1947							
<i>Eptesicus fuscus</i>	19		13	2			34
<i>E. marmotae</i> Farrell, 1956							
<i>Marmota monax</i>		128	3				131
<i>E. ohioensis</i> Farrell, 1956							
<i>Microtus pinetorum</i>		8			1	60	69
<i>Synaptomys cooperi</i>		25	1				26
<i>Sorex cinereus</i>					11		11
<i>E. peromysci</i> (Ewing, 1929)							
<i>Synaptomys cooperi</i>		248	51				299
<i>Peromyscus leucopus</i>	1	10	30			22	63
<i>Microtus pennsylvanicus</i>		19			20		39
<i>Microtus pinetorum</i>		13					13
<i>Zapus hudsonius</i>					12		12
<i>Marmota monax</i>		3					3
<i>Neotoma floridana</i>		2					2
<i>Tamias striatus</i>		2					2

TABLE II Numbers of chiggers taken from the various species of wild mammals of Indiana, summarized bimonthly. (For numbers of hosts examined, see Table I.) Continued

Host Species	Dec.- Jan.	Feb.- March	April- May	June- July	Aug.- Sept.	Oct.- Nov.	Totals
<i>Peromyscus maniculatus</i>						1	1
<i>Mustela frenata</i>					1		1
<i>Mustela nivalis</i>			1				1
<i>Ondatra zibethicus</i>					1		1
<i>E. pipistrelli</i> Brennan, 1947							
<i>Myotis lucifugus</i>				22	87		109
<i>Pipistrellus subflavus</i>					54		54
<i>Myotis keenii</i>					42	6	48
<i>Eptesicus fuscus</i>					3		3
<i>Myotis austroriparius</i>					1		1
<i>E. setosa</i> (Ewing, 1937)							
<i>Neotoma floridana</i>		6				49	55
<i>Microtus ochrogaster</i>		11					11
<i>Synaptomys cooperi</i>		5					5
<i>Sylvilagus floridanus</i>	3						3
<i>Peromyscus leucopus</i>		3					3
<i>E. trigenua</i> Loomis, 1956							
<i>Scalopus aquaticus</i>			3				3
<i>Leptotrombidium myotis</i> (Ewing, 1929)							
<i>Eptesicus fuscus</i>					2		2
<i>Leptotrombidium peromysci</i>							
Vercammen-Grandjean & Langston, 1976							
<i>Sciurus carolinensis</i>					14		14
<i>S. niger</i>					4		4
<i>Tamiasciurus hudsonicus</i>		2					2
<i>Zapus hudsonius</i>					1		1
<i>Microtrombicula trisetica</i> (Loomis & Crossley, 1963)							
<i>Sciurus carolinensis</i>					1		1
<i>Miyatrombicula cynos</i> (Ewing, 1937)							
<i>Sciurus niger</i>		2					2
<i>Tamiasciurus hudsonicus</i>		1					1
<i>Miyatrombicula jonesae</i> (Brennan, 1952)							
<i>Vulpes vulpes</i>		6					6
<i>Peromyscus leucopus</i>		1					1
<i>Neotrombicula fitchi</i> (Loomis, 1954)							
<i>Sciurus niger</i>	31		2			14	47
<i>Glaucornis volans</i>		40					40
<i>Tamiasciurus hudsonicus</i>	28						28
<i>Sciurus carolinensis</i>		13					13
<i>N. lipovskyi</i> (Brennan and Wharton, 1950)							
<i>Urocyon cinereoargenteus</i>						150	150
<i>Neotoma floridana</i>		1				43	44
<i>Microtus pinetorum</i>		3					3
<i>Microtus pennsylvanicus</i>		1					1
<i>Sylvilagus floridanus</i>						1	1
<i>N. microti</i> (Ewing, 1928)							
<i>Eptesicus fuscus</i>						4	4
<i>Procyon lotor</i>			4				4
<i>N. sylvilagi</i> (Brennan and Wharton, 1950)							
<i>Sciurus niger</i>						5	5

TABLE II Numbers of chiggers taken from the various species of wild mammals of Indiana, summarized bimonthly. (For numbers of hosts examined, see Table I.) Continued

Host Species	Dec.- Jan.	Feb.- March	April- May	June- July	Aug.- Sept.	Oct.- Nov.	Totals
<i>N. whartoni</i> (Ewing, 1929)							
<i>Sylvilagus floridanus</i>	36	29	4			90	159
<i>Urocyon cinereoargenteus</i>						1	1
<i>Didelphis virginiana</i>	2	12			1	122	137
<i>Sciurus niger</i>					6	62	68
<i>Vulpes vulpes</i>						40	40
<i>Tamias striatus</i>						23	23
<i>Sciurus carolinensis</i>						10	10
<i>Zapus hudsonius</i>						5	5
<i>Procyon lotor</i>						5	5
<i>Mustela vison</i>	1					1	2
<i>Mephitis mephitis</i>						1	1
<i>Microtus pennsylvanicus</i>						1	1
<i>Microtus ochrogaster</i>						1	1
<i>Walchia americana</i> Ewing, 1942							
<i>Sciurus niger</i>		2			41	22	65
<i>Sciurus carolinensis</i>	3				1	9	13
<i>Tamias striatus</i>						1	1

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Further Information on the Health and Longevity of the Gila Monster (*Heloderma suspectum* Cope)

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This short paper is a follow-up of a report on the occurrence of a melanoma in a captive specimen of the Gila Monster (Cooper, 1968). The reptile under study was obtained in August of 1945. Beginning in 1963 it developed a melanotic malignancy which was removed from the dorsal surface of the base of the tail on March 9, 1968. Procedures for the surgery and accompanying pathology were described in the above report.

The specimen under observation died October 8, 1977. It was taken to the laboratories of the department of pathology of Ball Memorial Hospital, Muncie, Indiana for post-mortem examination and pathological studies by Dr. Mark V. Braun and Dr. George E. Branam. Numerous kodachrome photographs were taken of both external and internal structures. Dr. Branam supervised the original studies in 1968.

The total length of the specimen was 510 mm as compared to 497 mm when the surgery was performed in 1968. Photographs were taken of the irregular hypopigmented scar from that surgery on the dorsal aspect of the tail. The scar measured 7 mm by 8 mm as compared to the 51 mm by 35 mm size of the original tumor. There were no other discernable superficial lesions on the animal. None of the thoracic or abdominal organs revealed gross evidence of primary malignancy. Biopsy samples were obtained from all organ systems and submitted for microscopic sections. Sections were also taken from the site of the original tumor. Microscopic examination demonstrated clusters of possible melanoma cells within the lymphatic structures of the lung, liver, and spleen. Dr. Branam felt that the cells were markedly similar to those of the original lesion, but the specific cause of death of the Gila Monster could not be determined from the examination findings, either gross or microscopic. There was little abdominal fat, which might reflect a state of malnutrition. The pigment-laden cells could represent an accumulation of melanin within the reticuloendothelial system as a normal process of aging.

The age of this reptile is of note. It was in captivity at Ball State University in the Biology Department for 32 years and 2 months. It was an adult of the same approximate size when received from Arizona in 1945. Bowler (1977) lists a November 1, 1975 record of *Heloderma suspectum* in the Little Rock Arkansas Zoo with an age of 27 years and 10 months. There is a second Gila Monster living at the present time in the Ball State University collection that was received also as an adult in August of 1945 and is still in good health. This appears to be the maximum known longevity for the species. A report has been sent to J. Kevin Bowler, Curator of Reptiles at the Philadelphia Zoological Gardens, of the ages of the above reptiles.

Kodachromes and microscopic sections are on file at the Ball Memorial Hospital Laboratories and at the Ball State University, Department of Biology.

Acknowledgment

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**A Study of a Population of
Passer domesticus that has been Shown
to be Carrying St. Louis Encephalitis**

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Abstract

A mark-recapture program was conducted at a stockyard on the northeast side of Seymour, Indiana. Blood samples taken from *Passer domesticus* at this site had indicated the presence of active St. Louis encephalitis virus. In 28 samples collected between June 30, 1977 and March 19, 1978, 1,068 *P. domesticus* were collected. Population parameters were estimated from the data applying a variation of Jolly's stochastic model. The very high population estimates and high proportion of juveniles in the population suggested that flocks of juveniles foraging in adjacent crop fields were being attracted to this urban site. The resulting high population density of this species may have played a significant role in the transmission cycle of St. Louis encephalitis at this site.

Introduction

In the summer of 1977, following a statewide epidemic of St. Louis encephalitis (SLE) in the summer of 1975 and a smaller outbreak in 1976, the Indiana State Board of Health initiated an arbovirus surveillance program. Blood samples were taken from *Passer domesticus*, the House Sparrow, in twenty counties across the state in order to identify viral activity. Sampling sites were selected on the basis of availability of large numbers of birds, the presence of suitable mosquito breeding habitat, and proximity to confirmed cases of SLE. Since SLE in the midwest is primarily an urban phenomenon, many of the sampling sites were located in urban areas. In light of what was understood about the ecology of *P. domesticus*, the use of urban sampling sites raised several objections. A description of the seasonal population ecology of this species is given by Summers-Smith (7). Man-made structures provide ideal nesting opportunities for this species and colonial nesting is common. Consequently, adult breeding populations of *P. domesticus* are centered in towns and cities. In late spring the first juveniles fledge and leave the nest. These juveniles do not remain in the towns and cities with their parents, instead they move into the surrounding crop fields to forage nomadically. These juveniles exhibit a strong flocking instinct and by midsummer large flocks containing several thousand birds may develop. As breeding ceases in the fall, the adults join the immatures in the rural areas, but even during this time the adults remain strongly attached to the nesting site, visiting it frequently. As winter sets in, all surviving individuals return to the towns and cities to overwinter. This species is known to be extremely sedentary and even the foraging juveniles usually never wander more than a few miles from their birthplace. The adults breeding in the urban areas are even more sedentary and probably never wander more than a few

thousand yards from their nests. Since urban populations are made up primarily of breeding adults with extremely restricted ranges, it was suggested that urban sampling sites were a poor choice since they only yielded information about the viral activity in a very small area. It was further suggested that this method did not yield any better coverage than a program employing sentinel flocks. Sentinel flocks are caged birds that are periodically checked for viral activity. Due to the number of man hours required to capture wild birds, sentinel flock programs are much less expensive to operate. In order to address these criticisms, an independent mark-recapture program was planned to look at the population dynamics of *P. domesticus* at one of these urban sites. The first serologic evidence of the presence of active SLE virus was obtained from a small stockyard on the northeastern edge of Seymour, Indiana. This was chosen as the study site.

Study Area and Methods

The study site was the Seymour Stockyard on the northeastern limit of Seymour, Indiana. The feed provided for the livestock served as the primary attractant for *P. domesticus*. The livestock shelters also provided a limited number of nesting sites. A bank of trees on the south side of the stockyard served as a communal roost and several residential homes in the immediate vicinity provided additional nesting sites. On the north and east, adjacent to the stockyard, were extensive crop fields planted primarily in corn (*Zea mays*). On the east side of the main shelter was a catch basin. In an attempt to identify mosquito breeding sites, Jackson County health officials obtained several samples of *Culex pipiens*, the presumed vector of SLE, from this empoundment.

Epidemiologic evidence also implicated this general vicinity as an area of potential viral activity. Of the 17 confirmed cases of St. Louis encephalitis in Jackson County since 1975, two of the victims lived within 1/4 mile of the stockyard and another 14 of the victims lived within one mile of it.

Between June 30, 1977 and March 19, 1978, 1,068 House Sparrows (*P. domesticus*) were captured. These included 335 birds captured by the State Board of Health. From these 291 blood samples were taken and 11 were found to be positive for SLE. Birds were captured in Japanese mist nets, 12 meters long and 2.6 meters high with a mesh size of 36 millimeters. These nets were placed so as to maximize the number of birds captured. Individual birds were banded with aluminum bands, size 1B, placed around the right tarsus. The age, sex, and time of capture of each individual was recorded.

Individuals were aged and sexed after a technique described by Johnston (3). However, immature birds which had not entered their post-juvenile molt were not sexed. Due to a persistence of the yellow coloration at the base of the mandibles, it was often possible to identify an individual as a first year bird even after it had acquired its adult plumage following the post-juvenile molt.

Population parameters were then estimated from the data using a modification of Jolly's stochastic model developed by Seber (6). A simple computer program was employed to speed calculations.

Results

Data

The data from 28 samples is summarized in Table I. Of the 1,068 birds captured, 1,005 were subsequently banded and released. There were two situations where captured birds were not banded. First, data collected by the Indiana State Board of Health arbovirus surveillance team is also included here. Blood samples were taken from these birds and a bird occasionally died as a result. The State Board of Health team also selectively bled juveniles and time requirements did not allow banding or sexing of adults. These birds are listed in Table I as sex unknown. In order to make their data useful to this study, the State Board of Health team did record the total number of captures.

Of the 1,005 birds banded, 144 were subsequently recaptured. This produced an overall recapture rate of 14.3%.

TABLE I *Banding Data for Seymour Stockyard*

Date	Captures				Recaptures				Total
	Males	Females	Juveniles	Unknowns	Males	Females	Juveniles	Unknowns	
* 6/30/77	6	8	7	0	0	0	0	0	21
7/9/77	6	3	24	0	0	0	0	0	33
* 7/14/77	0	0	34	0	0	1	0	0	35
7/17/77	8	5	32	0	0	0	1	0	46
** 7/21/77	1	4	14	6	2	0	2	0	29
7/24/77	7	5	76	1	0	0	8	0	97
** 7/28/77	0	0	20	14	0	0	3	0	37
** 8/4/77	0	0	22	14	0	0	3	0	39
* 8/11/77	4	1	12	0	1	0	3	0	21
8/13/77	1	0	74	0	0	0	2	0	77
* 8/18/77	0	0	18	0	0	1	1	0	20
** 8/25/77	0	0	18	10	0	0	3	0	31
8/27/77	0	0	68	1	0	0	12	0	81
9/10/77	1	2	122	1	0	0	15	0	141
* 9/15/77	0	0	18	0	0	0	3	0	21
* 9/21/77	0	0	8	0	0	0	3	0	11
* 9/28/77	0	0	19	0	0	0	6	0	25
10/2/77	3	0	40	1	1	0	5	0	50
10/16/77	3	1	15	0	0	0	8	0	27
*10/21/77	2	0	17	0	0	0	6	0	25
11/6/77	5	0	17	0	0	0	6	0	28
*11/16/77	3	0	6	0	0	0	5	0	14
11/19/77	17	1	57	0	2	0	20	1	98
1/6/78	2	0	7	0	2	0	5	0	16
1/22/78	4	0	11	0	2	0	3	0	20
2/26/78	1	1	2	0	1	0	1	0	6
* 2/28/78	2	2	0	0	2	0	0	0	6
3/19/78	5	3	1	0	1	1	2	0	13
Totals	81	36	759	48	14	3	126	1	1,068

* Collected by State Board of Health

** Samples selective for juveniles

Estimates of Population Parameters

A modification of Jolly's stochastic model for estimation of population parameters was applied to the data (6). The notation used in this discussion is consistent with Jolly's original notation (4), however, other authors referred to here have adopted their own notation. An excellent guide to equivalent notation is given by Cormack (2).

The Jolly method offers considerable advantages over previous models. This model may be applied to an open population, one in which there may be death, emigration, recruitment, or immigration. Any number of samples may be taken with varying intervals between samples. It is also not necessary to return all organisms to the population, thereby allowing for accidental death due to trapping or marking. Finally, it may be applied in the special case where entire samples are permanently removed from the population, as in commercial exploitation.

Before this model may be applied legitimately, the data must meet certain criteria. These requirements are:

1. The marked individuals become randomly mingled with the remainder of the population
2. Marked individuals are not affected by that marking
3. The two samples are both taken randomly or all individuals are equally available for capture with respect to mark status
4. The period of time taken during sampling must be small relative to the time interval between samples
5. Being captured does not affect the probability of an individual being subsequently captured.

The aluminum leg bands used in this study are those provided by the Fish and Wildlife Service. This is the preferred method since it is believed to have little or no effect on the survival of the bird.

Beimborn (1) showed that mist nets were not selective on the basis of sex and no evidence exists to suggest that they are selective on the basis of age. However, visual observations of the on site populations supported the estimated adult to immature ratios.

Finally, it was necessary to determine if the data from birds bled by the State Board of Health could be legitimately included in this study. If the bleeding reduced their survival probability significantly from that of the rest of the population, it would lead to an inflated estimate of population size. In light of the fact that a bird occasionally died from induced shock, it was necessary to study the problem more closely. In an attempt to determine any long term differences in survival between bled and unbled birds, the ratios of banded to recaptured birds were compared: 282 birds were bled, banded, and released; of these 53 were recaptured and 723 birds were simply banded; of these 91 were recaptured. This leads to a recapture rate of 18.8% and 12.6% respectively. This difference was not significant ($p > .01$).

In light of these considerations it is believed that this method may be legitimately applied. There is another consideration in applying Jolly's original

model: several of the samples contained a small number of recaptures. Fortunately, Seber (6) has developed a modification of Jolly's original method designed for small numbers of recaptures. It was this modified model that was used to estimate the population parameters in this study. Seber warns that even with the modified estimate, where recaptures are less than ten, the estimate may not even suggest the order of magnitude of the actual parameter and on this basis he suggests that samples where the number of recaptures is less than ten be deleted. However, since the degree of error is not only a function of the number of recaptures, but also a function of sample size, and since there is a reliable estimate of standard error, calculations for all samples have been made. However, certain estimates may be deleted from discussion on the basis of an unacceptably large standard error. There were also cases where variables placed in the variance formulae were evaluated as zero. Where this resulted in division by zero it was impossible to make an estimate of error. No estimate is shown in Table IV in these cases.

Trellis diagrams constructed after the method described by Jolly (4) are presented in Table II and Table III. The estimated population parameters and their standard errors are presented in Table IV. The parameters estimated after the method of Seber (6) are:

$\hat{N}_i$ = estimated number in the population when the i th sample is taken

$\hat{\phi}_i$ = estimated probability that an individual alive at the moment of release of the i th sample will survive until the time of capture of the $(i+1)$ th sample, including emigration and death

$\hat{B}_i$ = estimated number of new animals joining the population in the interval between the i th and the $(i+1)$ th samples and alive at time $i+1$.

Variance estimates for $\hat{\phi}_i$ and $\hat{B}_i$ were calculated after the method of Jolly (4) and the standard errors of the estimates were obtained by taking the square roots of these variances.

Seber (6) points out that Jolly's formula for the variance of $\hat{N}_i$,

$$\text{Var}(\hat{N}_i) = N_i(N_i - n_i) \left[\left(\frac{M_i - m_i + s_i}{M_i} \right) \left(\frac{1}{R_i} - \frac{1}{S_i} \right) + \frac{1 - \phi_i}{m_i} \right] + N_i - \sum_{j=0}^{i-1} \frac{N^2_i(j)}{B_j},$$

may be broken into two parts:

$$\text{A. } N_i(N_i - n_i) \left[\left(\frac{M_i - m_i + s_i}{M_i} \right) \left(\frac{1}{R_i} - \frac{1}{S_i} \right) + \frac{1 - \phi_i}{m_i} \right]$$

which estimates the variance due to errors of parameter estimation, and

$$\text{B. } N_i - \sum_{j=0}^{i-1} \frac{N^2_i(j)}{B_j}$$

which estimates variance due to stochastic fluctuation of the parameter.

Seber shows that the second element is negligible unless the proportion of the population banded is large. Since that is not the case here, the tedious

TABLE II *Trellis Diagram for Estimation of Z_i*

Sample Number																											
2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
0																											
0																											
1	1																										
0	0	1																									
1	2	2	4																								
0	1	2	5	8																							
0	0	0	0	0	3	3																					
0	0	0	0	0	3	4	4																				
0	0	1	2	2	3	4	4																				
0	0	0	0	0	0	0	0	2																			
0	0	0	0	0	0	0	0	2	2																		
0	0	0	0	0	0	0	0	2	3	3																	
0	0	0	0	0	2	2	2	3	10	11	12																
0	0	0	2	4	5	5	7	8	9	11	12	15															
0	0	1	3	6	7	7	8	9	9	11	12																
0	0	0	0	0	0	0	0	0	0	1	1	2															
0	1	1	1	1	2	2	2	3	3	3	3	3	3	3	3												
0	0	0	0	0	1	1	2	2	2	2	2	2	5	6	6	6											
0	1	1	2	2	2	2	2	2	2	2	2	4	4	4	6	6	6										
0	0	0	0	1	1	1	1	1	1	2	2	4	4	8	8	8	8	8									
0	1	1	1	1	2	2	2	2	2	2	2	2	2	4	4	5	5	5	6								
0	0	0	0	0	1	1	1	1	1	2	2	2	2	4	5	5	5	6	6								
0	0	0	0	0	0	1	1	1	1	2	2	2	2	4	5	5	6	6	6								
0	0	0	0	0	1	1	1	1	1	1	1	2	3	3	3	4	5	5	5	6							
0	0	0	0	0	0	1	1	1	1	1	1	2	3	3	3	4	5	5	5	5							
0	1	1	4	4	5	6	6	6	8	9	10	13	18	19	19	19	19	20	22	22	23						
0	0	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	4	5	5	6	7	7					
0	0	0	1	1	1	1	1	1	1	2	2	2	3	4	5	5	5	5	5	5	5	5	5				
0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	2	2	2	2	2	2	2	2	2		
0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	2	2	2	2	2	2	2	2	2	2	2		
0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	2	2	2	2	2	2	2	2	2	2	2		
0	0	0	0	0	0	0	0	0	1	1	1	2	3	3	3	3	3	3	3	3	3	3	3	3	3	4	
$Z_i =$	2	7	10	24	25	39	38	38	46	52	52	45	49	61	64	60	55	51	48	44	45	19	12	7	5	3	0

where:

Z_i = marked individuals before time i which are not caught in the i th sample but are caught subsequently

m_i = number of marked individuals in the i th sample

$3 = m_i$

TABLE III Trellis Diagram for Estimation of R_i

Date	n_i	s_i	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
6/30	21	20																												
7/9	33	33	0																											
7/14	35	32	1	0																										
7/17	46	46	0	0	1																									
7/21	29	23	1	1	0	2																								
7/24	97	94	0	1	1	3	3																							
7/28	37	23	0	0	0	0	0	3																						
8/4	39	23	0	0	0	0	0	3	0																					
8/11	21	18	0	0	1	1	0	1	1	0																				
8/13	77	76	0	0	0	0	0	0	0	0	2																			
8/18	20	20	0	0	0	0	0	0	0	0	2	0																		
8/25	31	21	0	0	0	0	0	2	0	0	1	0	0																	
8/27	81	79	0	0	0	2	2	1	0	2	3	1	0	1																
9/10	141	137	0	0	1	2	3	1	0	1	1	0	2	1	3															
9/15	21	21	0	0	0	0	0	0	0	0	0	1	0	0	1	1														
9/21	11	11	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0													
9/28	25	25	0	0	0	0	0	1	0	1	0	0	0	0	3	1	0	0												
10/2	50	50	0	1	0	1	0	0	0	0	0	0	0	0	2	0	2	0	0											
10/16	27	27	0	0	0	0	1	0	0	0	0	1	0	2	4	0	0	0	0	0										
10/21	25	25	0	1	0	0	0	1	0	0	0	0	0	0	0	2	0	1	0	0	1									
11/6	28	28	0	0	0	0	0	1	0	0	0	1	0	0	0	2	1	0	0	1	0	0								
11/6	14	14	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	1	1	0	0	0							
11/19	98	98	0	1	0	3	0	1	1	0	0	2	1	1	3	5	1	0	0	0	1	2	0	1						
1/6	16	16	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0						
1/22	20	20	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1	1	0	0	0	0	0	0	0					
2/26	6	6	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0				
2/28	6	6	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0			
3/19	13	13	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	
Total	1068	1005	2	6	4	18	9	17	2	4	10	8	3	5	19	15	6	2	1	4	3	2	1	2	0	0	0	0	1	= R_i

TABLE IV *Estimated Population Parameters*

Date	$\hat{N}_i$	Standard Error of $\hat{N}_i$	$\hat{\phi}_i$	Standard Error of $\hat{\phi}_i$	$\hat{B}_i$	Standard Error of $\hat{B}_i$
6/30			.486	.393	338.5	262.3
7/9	330.3	242.8	1.082	.684	1,335.7	812.7
7/14	1,663.2	1,001.3	.329	.163	64.9	581.9
7/17	604.8	601.7	.871	.271	-135.0	529.1
7/21	369.6	195.6	1.736	.592	923.5	626.2
7/24	1,523.8	646.9	1.394	.993	986.3	1,912.3
7/28	2,992.5	2,646.3	.553	.447	216.3	1,371.2
8/4	1,854.0	1,343.6	.339	.167	-312.9	380.7
8/11	306.4	149.6	1.729	1.877	8,924.6	7,875.5
8/13	10,284.4	8,066.0	.586	.368	-4,061.1	4,919.4
8/18	1,925.0	1,660.5	.661	.434	287.2	1,202.2
8/25	1,549.3	1,048.8	.907	.394	-171.2	825.9
8/27	1,211.0	413.5	1.680	.533	1,955.5	1,191.2
9/10	3,883.9	1,403.4	.348	.146	-246.4	662.6
9/15	1,070.9	683.7	1.218	.881	-507.4	852.6
9/21	777.0	628.7	2.944	3.440	662.4	1,655.0
9/28	2,919.4	3,062.4	.704	.763	2,091.3	2,019.2
10/2	4,131.0	2,578.9	.597	.429	-1,306.3	1,232.0
10/16	1,135.6	709.3	1.099	.951	346.2	704.6
10/21	1,567.4	1,211.8	1.460	1.740	413.6	1,319.6
11/6	2,668.0	2,818.9	.345	.403	-337.7	450.3
11/16	575.0	430.5	.966		3,382.8	
11/19	7,854.0		.107		-378.6	
1/6	448.4		.691		233.3	
1/22	532.0		.222		-27.1	
2/26	86.3		.305		4.7	
2/28	29.3		.242		4.6	
3/19	11.2		0		0	

calculation of part B of the formula was eliminated and the square root of part A of the formula was taken as the standard error of the estimate of $\hat{N}_i$.

Discussion

When making population estimates it is important to consider exactly what population it is you are measuring. In the case of this study any bird on site was available for capture, however no bird's movements were restricted to the sampling site alone. Thus, the population being measured was that of *P. domesticus* exploiting the sampling site. No spatial restrictions could be placed upon this population.

While the stockyard at Seymour covers only a little more than an acre, there were always a number of *P. domesticus* on site. During the winter as few as a dozen birds were observed there. The largest number of birds observed on site at one time was estimated to be near 200. While the standard errors of the population estimates of Table IV are too large to allow precise estimation of the size of this population, it is clear that this site was exploited by several thousand individuals in the course of the summer. This illustrates the problem inherent in population estimates based on observation alone.

The fact that the standard errors of the population estimates remained rather high throughout the sampling period, in itself suggests something about the population under study. If this were a population of breeding adults, there should have been little population turnover. Therefore, as banding continued throughout the study, a greater proportion of birds should have been banded after each sample. This should have been reflected by successively higher percentages of recaptures and lower standard errors for population estimates. Since this phenomenon is not observed, the population under study must have been extremely large in comparison to sample sizes and/or the population must have had a fairly high rate of turnover.

Another striking characteristic of this population was the tremendous fluctuation in population size over relatively short periods of time. To demonstrate this more vividly the population estimates of Table IV are graphed in (Fig. 1). It is clear that these fluctuations were too extreme to be accounted for by mortality and fecundity. Only very high rates of emigration and immigration could have accounted for the high rate of population turnover. This was also reflected by the very low values estimated for $\hat{Q}$ and the very high estimates for $\hat{B}_i$ (Table IV). Estimates such as these would only have been characteristic of an extremely mobile population. These results may at first appear to contradict the general opinion that *P. domesticus* is extremely sedentary. However, these results imply only that organisms entered or left the population exploiting the stockyard, and they say nothing about the total spatial distribution of the study population. Therefore, it is possible that organisms may have abandoned use of the study site in favor of other sites and still maintained a total range of only a few miles. This would have required abundant resources and very high population densities. The general area adjoining the study site easily could have provided these resource requirements, and high population densities are characteristic of this species. However, in light of these population estimates it is not possible to maintain that this was an urban nesting population. Adults are

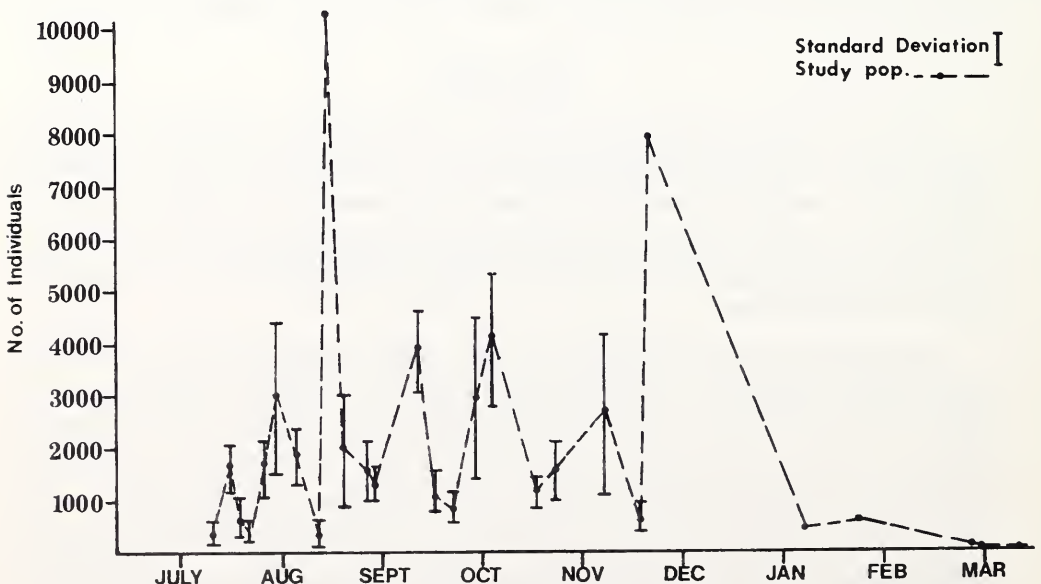


FIGURE 7.

much less mobile than juveniles and have a very strong attachment to the nesting site. The rate of population turnover was much higher than could reasonably have been expected for a nesting population. A great majority of the birds exploiting this site were no doubt recruited from flocks of juveniles in the adjoining fields. This assumption is supported by the very high ratio of juveniles to adults. Table V gives the percentage of adults and the percentage of juveniles captured in each sample along with the standard error of each of the percentage estimates. These statistics are graphed in (Fig. 2). Data from samples later than January were not used since first year birds become indistinguishable from adults of the year before. The age composition expected of a closed population was calculated from Will's composite model of population increase (8) and graphed in (Fig. 2) for comparison. While the highest juvenile to adult ratios occurred August through September just as the model predicts they should have, the extreme skew in favor of juveniles was much higher than could reasonably have been expected from the model. Considering the accepted scheme of seasonal dispersal, the proportion of juveniles at an urban nesting area should have been lower than predicted by Will's model. On the other hand, the proportion of juveniles in the outlying fields should have been higher than predicted by this model. On this basis it is suggested that a large complement of the population exploiting the stockyard was composed of *P. domesticus* recruited from foraging field flocks. Yet even if this is taken into consideration, the ratios were so skewed that the possibility of trapping selectivity for juveniles warrants consideration. Unfortunately, no work addressing this concern exists.

TABLE V *Proportions of Adults and Juveniles in the Study Population*

Date	Percentage of Adults	Percentage of Juveniles	Standard Error
6/30/77	66.7	33.3	10.3
7/9/77	27.3	72.7	7.8
7/17/77	28.3	71.7	6.6
7/24/77	12.5	87.5	3.4
8/11/77	28.6	71.4	9.9
8/13/77	1.3	98.7	1.7
8/18/77	5.0	95.0	4.9
8/27/77	0.0	100.0	1.1
9/10/77	2.1	97.9	1.2
9/15/77	0.0	100.0	2.2
9/21/77	0.0	100.0	3.0
9/28/77	0.0	100.0	2.0
10/2/77	8.2	91.8	3.9
10/16/77	14.3	85.9	6.6
10/21/77	8.0	92.0	5.4
11/6/77	11.6	82.1	7.2
11/16/77	21.4	78.6	11.0
11/19/77	20.6	79.4	4.1
1/6/78	25.0	75.0	10.8

Calculated from TABLE I. Samples taken after January and samples which were selective for juveniles are not included.

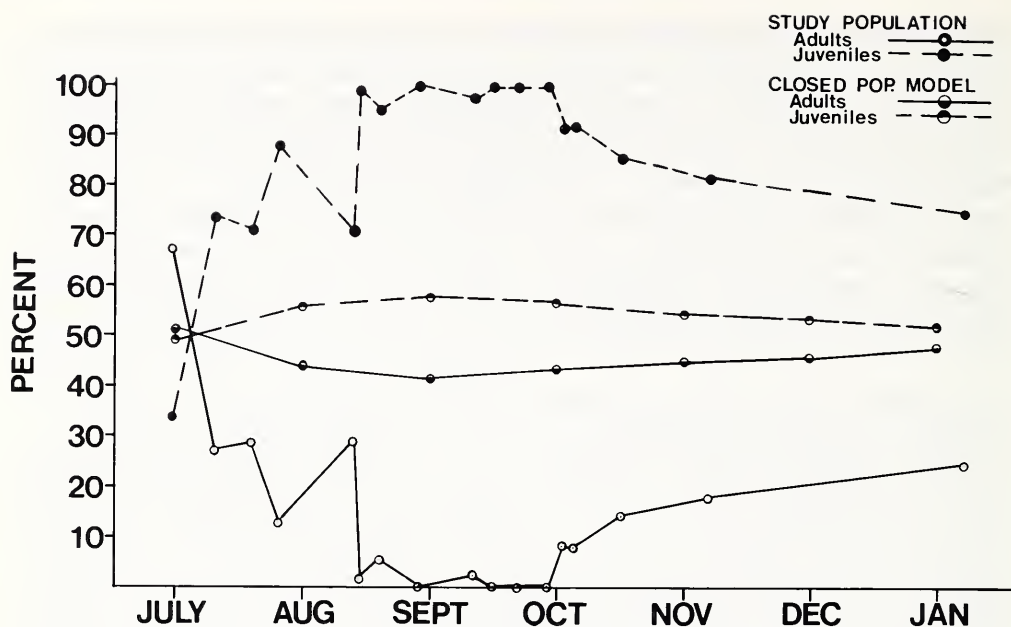


FIGURE 2.

Juveniles from the crop fields were probably attracted to the study site by the availability of livestock feed. However, if juveniles were attracted to the study site only during the day to feed, they could not have become involved in the transmission cycle of SLE, since *Culex pipiens*, the presumed mosquito vector, is a nocturnal feeder. It is therefore of significance that juveniles also seemed to be attracted to this site by the bank of trees on the south side of the study site which they used as a nocturnal roost. Very few roosting sites were available in the crop fields in the surrounding area and no doubt many juveniles were forced to return to town to roost at night. While *P. domesticus* normally roost in the crop fields if roosting sites are available, daily movements back to town for roosting are not unknown. North (5) briefly mentions observing this phenomenon in Oklahoma.

This daily dispersal pattern would have resulted in very high densities of birds at the study site during a critical period. It seems possible that these high densities may have contributed to the amplification of SLE virus in this area. This relationship clearly warrants future investigation.

The data collected in this study also suggests that urban sampling sites which exhibit high juvenile to adult ratios are preferable to other urban sites and this may be used by arbovirus surveillance personnel as a criterion for selecting future sampling sites.

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Chemotactic Responses of *Halteria grandinella*

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Introduction

Chemotactic studies form a relatively recent field and have their origin in the work of Pfeffer and Englemann (2). Since that time considerable work has been done with a variety of microorganisms, including bacteria and protozoa.

Diehn (1), working with *Euglena gracilis*, concluded that the organism exhibited both positive phototactic and inverse photophobic responses. He hypothesized that some single-celled organisms utilize homeostatic systems for orientation movements. Seravin and Orlovskaja (3) utilized six species of carnivorous protozoa in an investigation of the behavioral activity involved in feeding responses. Tamar (5) reported on the reactions of *Halteria grandinella* to a variety of substances, including KCl, CaCl₂, distilled water and acetic acid.

Previous work by Tamar (4, 5, 6, 7) supports the feasibility of using *Halteria grandinella* for chemotactic studies. It is a phylogenetically advanced ciliate protozoan possessing several different types of movement and a relatively short response time. The last property enables one to maximize testing periods.

The purpose of this experiment was to determine a series of chemotactic responses of *Halteria grandinella*.

Materials and Methods

The tests were run with different time periods for each substance, ranging from 15 to 120 minutes. However, in the case of the substances eliciting weak or strong positive responses, the reaction of the *Halteria* was maximal within 25 to 35 minutes. The optimal time for any *Halteria* response was determined from original tests. The temperature during all the tests never exceeded 23°C or dropped lower than 20°C. The pH of the test substance, whenever possible, was made equal to that of the culture medium. A specific medium was used to culture and test the *Halteria* (6).

Five major test methods were used in the course of the research. The first method involved the use of a petri dish cover or bottom. A smear of the test substance was dried in the dish and a lake of *Halteria*-filled culture fluid was then added. In the next method a lake was also employed, except that the lake was much smaller and extended over a slide smear. A third method again required a petri dish lake. However, in this case a capillary with a specific amount or concentration of the test substance was inserted into the lake. In addition to the petri dish-capillary method a slide-capillary method was also tried. Further, a slide with a coverslip held up by an empty capillary and one filled capillary was used. The final method, and the most effective, provided the best points of all the other methods. A slide with a tiny dried smear in one or two

areas was covered with a lake. Two long pieces of thread were located at either end of the lake and a coverslip was placed on top of the lake and was held up by the thread.

The slide and petri dish tests were observed with a binocular dissecting microscope at a magnification of 10 to 70X. The light bulb serving as the light source was covered by 3 heat filters which effectively prevented any significant heat transmission. A phase contrast microscope was used mainly for identification of the *Halteria* and some of the test bacteria and mold.

Results

In the following tables the responses of the *Halteria* are grouped into five categories. These are: strongly positive, weakly positive, neutral, weakly negative and strongly negative. In a strongly positive response, the *Halteria* in a test are found almost exclusively over and around the test substance. A weakly positive response is a gathering of *Halteria* around and over the test substance, but *Halteria* are in other places of the test area also. A neutral response consists of an even distribution of the *Halteria* over the total test area. A weakly negative response is the even distribution of *Halteria* in most of the test area, but there are fewer over or around the test substance. Finally, a strongly negative response is the total avoidance by the *Halteria* of the substance placed in the test area.

The symbols that are used for these responses are: strongly positive = +, weakly positive = 0, neutral = 0, weakly negative = 0, and strongly negative = -. The symbols used for the methods are the following: lake-petri dish = 1, capillary-in-a-lake = 2, lake-on-a-slide = 3, capillary-on-a-slide plus a coverslip = 4, smear-on-a-slide with a coverslip held up by thread = 5. An example of a recorded response may read 03, +2, 01. This means 3 tests were weakly negative, 2 tests were strongly positive and 1 test was neutral.

This research was particularly directed at finding substances that elicit a positive response from *Halteria grandinella*. Many substances produced semi-positive or neutral responses. However, only three initiated a high percentage of strongly positive responses by the test organisms. These three substances are: a string soaked in skimmed milk, dried bacterial film and cultured water mold grown in a solution at a pH of 7.

Most of the hundreds of tests that were run produced a negative response from the *Halteria*. A neutral response by the *Halteria* was also common. Those substances out of over 75 that produced any kind of positive response are listed in the following tables.

All the substances listed in Table I were tested after they were brought to a pH of 7 and the culture medium was either at pHs between 6.8 and 7.2 or was also brought to pH 7.

Table II gives the results obtained with 7 amino acids that elicited a highest number of weakly positive responses when the medium was at pHs between 6.8 and 7.2 or was brought to pH 7 and the amino acids were at lesser (and their natural) pHs. These lesser pHs were the following: isoleucine 6.0, leucine 6, serine 5.7, proline 6.3, cystine 5.0, methionine 5.7 and tyrosine 5.6. When each of these amino acids was tested 10 times as above, with the exception that the pH of

TABLE I *A listing of the items tested at a pH near or at neutral.*

Item tested	Response	Method
albumen (raw)	010, +1	1
unwashed water mold	019, 014, 02	1
bacteria (cocci?) from pond water (2 yellow colonies/10 ml of culture fluid)	09, 08, 03	1
water mold and riboflavin	08, 07, +4, 01	2
cultured water mold	+5, 03, 02	5
powdered skimmed milk	030	2
string soaked in skimmed milk	+7, 06, 06, 01	1

TABLE II *A listing of amino acids tested at lower than neutral pHs.*

Item tested	Response	Method
isoleucine L(.125m)	024, 023, +1, -1, 01	2
isoleucine D(.125m)	09, 03, -3	5
leucine D(.125m)	06, 05, 03, -1	5
serine L(.125m)	05, 04, +3, 01	5
proline L(.125m)	07, -5, 03	5
cystine L(.125m)	09, -5, 02	5
methionine L(.125m)	011, 04	5
leucine DL(.125m)	06, 05, 04	5
serine L(.375m)	015, 05	5
cystine L(.375m)	015, 03, 02	5
tyrosine L(.375m)	05, 04, 01	5

the amino acids was brought to 7, predominantly neutral responses were obtained.

Table III lists all the substances that elicited a positive response from the *Halteria* but were at unknown pHs. The medium was always at a pH between 6.8 and 7.2 in the Table III tests.

TABLE III *A listing of the items tested that elicited a positive response, but at an unknown pH.*

Item tested	Response	Method
water plant stem (cut)	07, +2, 02	1
dried pond water	011, 07, 01, -2	3
whole midge	05	1
whole raw egg	011, 08, +2, 01	1
mealworms (cut)	012, 011, -2	5
fly larvae (cut)	014, 011	5
dried bacterial film	+8, 08, 02, 02	3

Discussion

Since skimmed milk powder is added to the culture medium it is possible that skimmed milk, bacteria and water mold may have to be present together to elicit a large proportion of positive responses from the *Halteria* with the experimental procedures employed in the present research. It may also be that bacteria attracted to the skimmed milk may in turn have attracted *Halteria* during the 25-35 minutes used in the skimmed milk tests.

The data showed that of all the 15 amino acids tested at their natural, lower than 7, pH, only the 7 given in Table II produced some kind of positive response and this positive response was very weak. The fact that each of these same 7 amino acids gave mainly neutral responses when it was brought to a pH of 7 indicates that the acidic nature of these amino acids may have been largely responsible for the attraction.

The other items that produced positive responses are logical choices for testing in view of the natural environment of the *Halteria*. Such items as a whole midge, mealworms and fly larvae, as well as pond water bacterial colonies and water mold, may be present in the pond water inhabited by *Halteria*. The preceding items plus egg, water plant stem and powdered skimmed milk all may have one factor in common. Each of them could offer the *Halteria* a concentration of food bacteria and perhaps also additional nutrients.

It should be remembered that skimmed milk, bacteria, and sometimes water mold were present in the culture medium, and this may have reduced the strength of the chemotactic responses.

Studies of the responses of *H. grandinella* to several amines and other nitrogenous substances are in progress.

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